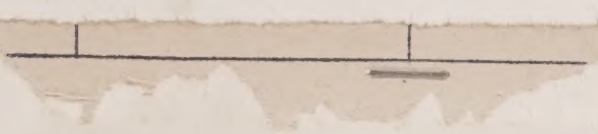
L 82 4p
N 56



SECOND INTERNATIONAL SEAWEED SYMPOSIUM

Second International Seaweed Symposium

(Held in Trondheim, July 1955)

Edited by

TRYGVE BRAARUD & N. A. SØRENSEN

PERGAMON PRESS
LONDON & NEW YORK
1956

FP 3770
I, 22"/p
N56

Published by Pergamon Press Ltd.
4 & 5 Fitzroy Square, London W.1
and Printed by Robert MacLehose and Co. Ltd.
The University Press, Glasgow

CONTENTS

List of active members *page* ix
Preface xiii

I. CHEMICAL COMPOSITION OF SEAWEEDS

X-Ray and Infrared Studies on Carrageenin . . . *by* S. T. Bayley 3
Water Soluble Polysaccharide of *Cladophora rupestris* . . . *by* I. S. Fisher and E. E. Percival 5
Seasonal Variation in Chemical Composition of *Laminaria digitata* from Different Parts of the Norwegian Coast . . . *by* A. Haug and A. Jensen 10
Carotene Content of some Norwegian Seaweeds, and Observations on the Breakdown of Carotene in Seaweeds and Seaweed Meal . . . *by* A. Haug and B. Larsen 16
Some Constituents of *Porphyridium cruentum* . . . *by* F. T. Haxo and Colm O'h Cocha 23
Postmortal Changes in the Carotenoids of *Fucus vesiculosus* . . . *by* S. Liaaen and N. A. Sørensen 25
Low-molecular Weight Carbohydrates in Brown and Red Algae . . . *by* B. Lindberg 33
On the Occurrence of Vitamins in Marine Algae . . . *by* H. Lundin and L.-E. Ericson 39
Variations in the Chemical Composition of Spanish Brown Seaweeds . . . *by* C. Primo 44
Studies on the Heterogeneity of Carrageenin . . . *by* David B. Smith, A. N. O'Neill and A. S. Perlin 45

II. STUDIES WITH SPECIAL REFERENCE TO PRACTICAL USES

The Production of Antibiotic Substances by Seaweeds . . . *by* C. G. C. Chesters and J. A. Stott 49
Sodium Laminarin Sulphate as a Blood Anticoagulant . . . *by* E. T. Dewar 55

The Vitamin Content of Algae and its Scientific Consequences . . . *by* POUL FREUDENTHAL *page* 62

A Study of Seaweed Drying . . . *by* R. G. GARDNER and T. J. MITCHELL 63

Experiments with Seaweed Meals as Supplements to Rations for Chicks and Laying Hens . . . *by* J. HØIE and Ø. SANDVIK 82

Alginyl Stearate . . . *by* L. DE KEYSER 98

Seaweed for Stock Feeding . . . *by* A. A. MCINNES 103

Production of Mannitol and Laminarin from Seaweed Aqueous Extracts . . . *by* KENNETH C. REID 105

The Use of *Macrocystis pyrifera* as Source of Trace Elements in Human Nutrition . . . *by* G. L. SEIFERT and H. C. WOOD 107

The Iodine Content of Eggs from Hens fed with Seaweed Meal . . . *by* I. SUNDE 109

A Comparison of the Properties of Various Preparations of Sodium Alginate . . . *by* D. L. VINCENT, D. A. I. GORING and E. GORDON YOUNG 114

III. METHODS FOR ANALYSES AND EXPLOITATION

On the Detection of Alginic Acid in Tissues by Means of Birefringence . . . *by* GEERT ANDERSEN 119

On the Quantitative Determination of Alginic Acid . . . *by* A. JENSEN and I. SUNDE 125

The Detection of Small Quantities of Alginates . . . *by* R. H. MCDOWELL 131

IV. MICROBIOLOGY

Decomposition of Laminarin by Micro-organisms . . . *by* C. G. C. CHESTERS, M. TURNER and A. APINIS 141

The Enzymatic Hydrolysis of Carrageenin . . . *by* W. YAPHE and BEVERLEY BAXTER 145

V. BOTANICAL STUDIES OF SEAWEEDS

A Quantitative Study of the Benthic Communities inhabiting the Kelp Beds off the California Coast, with a Self-contained Diving Apparatus . . . *by* ANWAR ABDEL ALEEM *page* 149

The Growth Rings in *Alaria* Stipes . . . *by* EGIL BAARDSETH 153

Observations on the Time of Reproduction and the Rate of Growth of Certain *Fucaceae* . . . *by* HELEN BLACKLER 158

Growth Control in the *Fucaceae* . . . *by* E. M. BURROWS 163

Seaweeds of Economic Importance in South China . . . *by* BEK-TO CHIU 171

The Ecology of *Gracilaria confervoides* (L.) Grev. in South Africa with Special Reference to its Ecology in the Saldanha-Langebaan Lagoon . . . *by* WM. EDWYN ISAAC 173

Studies in Seaweed Populations of Economic Importance in Digby Neck Area, Nova Scotia . . . *by* CONSTANCE I. MACFARLANE 186

Cytological Observations on three British Species of *Laminaria*; a preliminary report . . . *by* MARGARET NAYLOR 192

Recuperation and Recolonisation in *Ascophyllum* . . . *by* HENRIK PRINTZ 194

Phenology of *Ascophyllum nodosum* . . . *by* HENRIK PRINTZ 198

Perennial Changes of *Laminaria cloustoni Edm.* (*L. hyperborea* (Gunn.) Fosl.) around Scotland . . . *by* W. D. RICHARDSON and F. T. WALKER 203

The Resistance of *Gracilaria confervoides* to Hydrogen Sulphide . . . *by* KNUT STOKKE 210

Projet de Cartographie des Plantes Marines utiles à l'Industrie . . . *par* AD. DAVY DE VIRVILLE 215

On the Distribution of Sargassum on the Coast of Japan and its Neighbouring Regions . . . *by* Y. YAMADA 218

LIST OF ACTIVE MEMBERS

Aleem, Anwar Abdel, Professor, University of Alexandria, Alexandria, Egypt.

Andersen, Geert, Dr., Kolloidchemische Abteilung der Universität Kiel, Wertring 211, Kiel, Germany.

Aravio-Torre, Jesús, Dr., Alcala 27, Madrid, Spain.

Baardseth, Egil, Dr., Norsk institutt for tang- og tareforskning, N.T.H., Trondheim, Norway.

Bakken, Kåre, Mr., Gjöaveien 46, Bergen, Norway.

Becker, Charles Robert, Mr., Vegemar SA, Immeuble C.T.M., 23, Rue Léon l'Africain, Casablanca, Maroc.

Bergmann, Werner, Professor, Yale University, New Haven, Conn., U.S.A.

Bernhard, Michael, Mr., Food and Agricultural Organization, Viale delle Terme di Caracalla, Roma, Italy.

Björnsson, Hallgrimur, Mr., Flókagata 12, Reykjavik, Iceland.

Blackler, Helen, Dr., The Gatty Marine Laboratory, The University, St. Andrews, Fife, Scotland.

Braarud, Trygve, Professor, Institutt for marin biologi, avd.B, University of Oslo, Oslo-Blindern, Norway.

Burrows, Elsie, Dr., Hartley Botanical Laboratories, The University, Liverpool 3, England.

Chalklin, Kathleen, Miss, Department of Botany, University Science Laboratories, South Road, Durham, England.

Chamberlain, Yvonne, Miss, Botany Department, British Museum (Nat. Hist.), Cromwell Road, London S.W.7, England.

Chesters, Charles G. C., Professor, Department of Botany, University of Nottingham, Nottingham, England.

Chiu, Bek-To, Miss, University of Hong Kong, Department of Biology, Hong Kong.

Christiansen, Erik, Mr., I/S Algadan, Fredrikshavn, Denmark.

Conway, Elsie, Dr., Department of Botany, The University, Glasgow, Scotland.

Dano, Jean Henri Marie, Mr., Satia, 104 Rue des Champs Elysées, Paris, France.

Dewar, Eric Thomas, Dr., Institute of Seaweed Research, Inveresk, Midlothian, Scotland.

Diannelidis, Themistokles, Professor, Botanical Institute, University of Thessaloniki, Thessaloniki, Greece.

Egli, Robert, Dr., Afico, S.A., La Tour de Peilz, Switzerland.

Flood, Donal T., Research Director, Institute for Industrial Research and Standards, Glasnevin House, Ballymun Road, Dublin, Ireland.

Freudenthal, Poul, Chief-Surgeon, M.D., H.C. Ørstedsvej 10, København, Denmark.
Gardner, Robert G., Dr., Technical Chemistry Department, Royal Technical College, Glasgow C.1, Scotland.
Glynn, Charles D., Mr., Irish Marine Products Ltd., Kilrush, Ireland.
Goodspeed, T. Harper, Professor, Department of Botany, University of California, Berkeley 4, Cal., U.S.A.
Grenager, Birger, Mr., Norsk institutt for tang- og tareforskning, N.T.H., Trondheim, Norway.
Halsall, Redfern, Mr., 49 Rodney Street, Liverpool 1, England.
Hatlebrekke, Fridtjov, Mr., A/S Ellingsöya Fiskemelfabrikk, Ålesund, Norway.
Haug, Arne, Mr., Norsk institutt for tang- og tareforskning, N.T.H., Trondheim, Norway.
Haxo, Francis, Dr., Scripps Institution of Oceanography, University of California, La Jolla, Cal., U.S.A.
Heaton, John, Dr., Tame House, Reddish Vale, Stockport, Cheshire, England.
Heen, Øystein, Mr., Industridirektoratet, Skippergata 33, Oslo, Norway.
Hoffmann, Curt, Professor, Institut für Meereskunde, Hohenbergstr. 2 Kiel, Germany.
Høie, Johs., Professor, Norges landbrukshøgskole, Vollebekk, Norway.
Isaac, William Edwyn, Professor, Department of Botany, University of Cape Town, Rondebosch, Cape, South Africa.
Jangaard, P. M., Mr., Fisheries Experimental Station, Halifax, N.S., Canada.
Jensen, Arne, Mr., Norsk institutt for tang- og tareforskning, N.T.H., Trondheim, Norway.
Jorde, Ingerid, Miss, Ullevålsveien 35, Oslo, Norway.
Keijser, Louis Felix de, Mr., Gevaert Photo-Production N.V., Mortsel (Antwerp), Belgium.
Koster, Jósephine Th., Dr., Rijksherbarium, Nonnensteeg 1, Leiden, Holland.
Kristensen, Kåre, Mr., A/S Protan, Drammen, Norway.
Kylin, Elsa, Fil.mag., St. Södergatan 4, Lund, Sweden.
Lande, Frans van der, Mr., Venette, B. post. 52, Compiègne, France.
Larsen, Bjørn, Mr., Norsk institutt for tang- og tareforskning, N.T.H., Trondheim, Norway.
Lee, Eilis, Miss, Chemistry Department, University College, Galway, Ireland.
Lienau, H. C., Mr., Am Pferdewasser 4, Flensburg, Germany.
Lindberg, Bengt, Dr., Institutionen før organisk kemi, Kungl. Tekniska Högskolan, Stockholm, Sweden.
Lundin, Harry, Professor, Kungl. Tekniska Högskolan, Stockholm, Sweden.
Lærdal, Arnbjørn, Mr., Frøystad Forsøksgård, Gausel, Jæren, Norway.

McDowell, Richard Henry, Mr., Alginate Industries Limited, Walter House, Bedford Street, Strand, London, England.
McInnes, Alexander Archibald, Mr., Moray Firth Seaweed Products Ltd., Geddes, Nairn, Scotland.
Megias, Jacinto, Dr., Universidad de Madrid, Facultad de Farmacia, Ibánez Martin 4, Madrid, Spain.
Moss, Betty, Dr., Department of Botany, King's College, Newcastle-upon-Tyne, England.
Naylor, Margaret, Dr., Department of Botany, The University, Hull, England.
Newton, Lily, Professor, University College of Wales, Aberystwyth, Wales, U.K.
Percival, Ethel Elizabeth, Dr., Chemistry Department, University, Westmains Rd., Edinburgh, Scotland.
Perelló, J. M., Professor, Universidad de Madrid, Facultad de Farmacia, Ibánez Martin 4, Madrid, Spain.
Powling, Philip, Mr., Paines & Byrne Ltd, Pabyrn Laboratories, Greenford, Middlesex, England.
Reid, Kenneth C., Mr., Institute of Seaweed Research, Inveresk, Midlothian, Scotland.
Richardson, Dudley, Mr., Imperial College of Tropical Agriculture, Trinidad, The British West Indies.
Rodriguez, Olegario, Mr., Direccion General de Pesca Maritima, Alarcón 1, Madrid, Spain.
Romijn, Christiaan, Professor, Laboratorium voor Veterinaire Physiologie der Rijks Universiteit, Utrecht, Holland.
Ruaidh, Seán Ó, Mr., Department of Lands, 34 Westland Row, Dublin, Ireland.
Ruebush, Trenton K., Commander U.S.N., Office of Naval Research, 429 Oxford St., London W.1, England.
Schmidt, Ernst K. H., Mr., Kl. Stuebeheide 40, Hamburg-Fu. 1, Germany.
Schulz, Alfred Paul, Dr., Stern Str. 27, Düsseldorf, Germany.
Seifert, George L., Mr., 1503 Foulkrod St., Philadelphia 24, Pa., U.S.A.
Smayda, Theodore John, Mr., Narrangansett Marine Laboratory, University of Rhode Island, Kingston, Rh.I., U.S.A.
Smith, Gordon Gregor, Mr., Botany Department, University of Western Australia, Nedlands, West. Australia.
Smith, Ingolf, Mr., Statens Landbrukskjemiske Kontrollstasjon, Pilestredet 27, Oslo, Norway.
Stokke, Knut, Mr., Institutt for marin biologi, avd.B, Oslo-Blindern, Norway.
Storleer, Johs., Mr., Harald Løvenskiolds vei 19, Røa, Oslo.
Sundene, Ove, Dr., Botanisk museum, Oslo 45, Norway.
Sutphen, William T., Dr., Stanford Research Institute, Stanford, Cal., U.S.A.

Sørensen, Nils Andreas, Professor, Institutt for organisk kjemi, N.T.H., Trondheim, Norway.

Sørensen, Jørgine Stene, Mrs., Institutt for organisk kjemi, N.T.H., Trondheim, Norway.

Torgersen, Haakon, Mr., Algea Produkter A/S, Kristiansund N, Norway.

Torgersen, Hans H., Mr., Algea Produkter A/S, Kristiansund N, Norway.

Townsend, Henry Horace, Mr., Brook Lodge School Hill, Chedale, Cheshire, England.

Valéra, Máirín de, Dr., Coláiste Ollscoile, Gaillimh, Ireland.

Veyhe, Jacob, Mr., Tórshavn, Faeroe Islands.

Virville, Adrian Davy de, Dr., Faculté des Sciences, Université de Paris, 12 Rue Cuvier, Paris 5, France.

Weingand, Richard, Mr., Oskar Wolff Strasse 18, Walsrode, Germany.

Wood, Curtis H., Mr., 1503 Foulkrod Street, Philadelphia 24, Pa., U.S.A.

Woodward, F. N., Dr., Institute of Seaweed Research, Inveresk, Midlothian, Scotland.

Wœrn, Mats, Dr., Växtbiologiska Institutionen, Uppsala, Sweden.

Yamada, Yukio, Professor, The Institute of Algological Research, Faculty of Science, Hokkaido University, Sapporo, Japan.

Young, E. Gordon, Dr., Maritime Regional Laboratory, National Research Council, Oxford Street, Halifax, Canada.

In addition to the 90 active members, 34 accompanying members were present.

PREFACE

THE First International Seaweed Symposium in Edinburgh in 1952, sponsored by the Institute of Seaweed Research, represented an attempt to co-ordinate on an international basis the work in seaweed research aiming at industrial exploitation.

Acting upon a suggestion made at this symposium to conduct a similar symposium at a later date, the Norwegian Institute of Seaweed Research issued invitations to a second symposium in Trondheim, which was held on 14 to 17 July 1955.

The organizing committee consisted of Professor TRYGVE BRAARUD, University of Oslo (chairman), Mr. BIRGER GRENAGER, Norwegian Institute of Seaweed Research (secretary), Mr. ARNE HAUG, Norwegian Institute of Seaweed Research, and Professor NILS ANDREAS SØRENSON, Norwegian Technical University, Trondheim. The committee was assisted by other members of the staff of the institute, who also took part in compiling abstracts of the discussions.

The organizing committee indicated a scope for the symposium which would bring together not only specialists from various scientific fields, but representatives of the various practical approaches of seaweed exploitation as well. The reports presented, therefore, cover a variety of fields.

An obvious necessity of basic biochemical research in order to establish a sound scientific basis for the present and future uses of the seaweed resources of the world formed the background for many of the chemical studies on seaweeds which were reported. Experience in areas where seaweeds are extensively harvested have proved that special botanical studies are needed in order to obtain estimates of available yields over a long period, and to plan measures for a rational conservation of the stock. In addition to the papers of a more general biochemical and botanical nature, the programme included reports of special interest to those using seaweeds commercially, such as descriptions of analytical procedures, feeding experiments with seaweed products, and handling of the raw material for industrial purposes.

Some of the communications are extensive reports; others have the character of abstracts, the main data being published elsewhere. In both cases the papers are published in their original form, without alterations in phrasing.

The editors wish to express their gratitude to Pergamon Press Ltd for having accepted the proceedings for publication, to the authors for their kind co-operation, and to Mr. BIRGER GRENAGER and other members of the staff of the Norwegian Institute of Seaweed Research for their assistance in preparing this volume.

TRYGVE BRAARUD
NILS ANDREAS SØRENSEN

Section I

CHEMICAL COMPOSITION OF SEAWEEDS

X-RAY AND INFRARED STUDIES ON CARRAGEENIN

By S. T. BAYLEY (Canada)

Read by E. GORDON YOUNG (Canada)

ABSTRACT

X-RAY diffraction studies on whole carrageenin and its κ- and λ-components have established structural differences between the two components and shown that the fibre repeat period in all three materials is 25·2 Å. The κ-carrageenin molecule is probably branched. The main chain appears to consist of residues of sulphated D-galactose linked 1,3- and 3,6-anhydro-D-galactose linked 1,4- in the ratio 2 : 1. At intervals of 25·2 Å along this chain, single side residues of 3,6-anhydro-D-galactose seem to be attached, probably through carbon 6 of a sulphated residue. The λ-carrageenin molecule appears to be composed mainly of sulphated D-galactose residues linked 1,3-, together with some non-sulphated residues, whose distribution either within the main chain or as side chains probably accounts for the 25·2 Å period. The infrared spectra of κ- and λ-carrageenin are similar. An explanation of the potassium sensitivity of κ-carrageenin is offered in terms of its proposed structure.

DISCUSSION[1]

Dr. ISAAC, South Africa, asked Dr. YOUNG if the observations could allow any conclusions to be drawn concerning the relationship between particular physiological events and the occurrence of the two carrageenins. Dr. YOUNG felt with considerable certainty that the amounts of the components relative to each other varied throughout the year. Extraction experiments had shown that at certain times of the year they got almost nothing but the λ-form; especially in the summer though, they found a little more of the κ-form. In other species than *Chondrus* the author knew that the proportion of the carrageenin components varied throughout the year.

Dr. LINDBERG, Sweden, found it difficult to see how hydroxymethyl-

[1] Refers also to the paper by SMITH, O'NEILL and PERLIN, p. 45.

furfural could be formed from a 3,6 anhydrogalactose unit as he considered the 3,6 oxygen ring as a quite stable arrangement to acid hydrolysis.

Dr. Young replied that in so far as Dr. O'Neill had been able to isolate the anhydro galactose he had had great difficulties because of its great instability. The amounts of the anhydro sugar were determined by the furfural formation. Dr. Lindberg wanted to know if they had actually isolated the anhydro sugar and shown that this could give rise to the hydroxymethylfurfural, to which Dr. Young stated that this had been done.

Dr. Percival, Scotland, asked how the ratio of the anhydro sugar to the furfural was determined to be 1·4 to 1. Dr. Young said that this had been done by isolation and hydrolysis and by determination of the hydroxymethylfurfural in the Beckman DU spectrophotometer. Dr. Percival said that she had done the same experiment and that she was not quite convinced that this procedure would give absolutely correct results. She wanted to know if the proportion had been determined by any other means, but Dr. Young did not know of any.

Mr. Jensen, Norway, asked if Dr. Young had any information on the possibility of a transformation of λ to κ-carrageenin to which Dr. Young said that evidence was lacking.

Dr. Andersen, Germany, wanted to know if both the carrageenin forms were present as such in the algae and had not been formed during preparation. He drew attention to the fact that the properties of pectins are dependent on the method of extraction. Dr. Young answered that extractions at a series of different temperatures had always given the two carrageenins so they were reasonably certain in stating that they had at least these two types. Each of these two types might in their own way be heterogeneous from the standpoint of molecular weight. The κ-form was supposed to be approximately 300,000 and the λ-form 600,000 but they had obtained κ-forms running up to over one million determined by light scattering techniques. So he would not say they had two homogeneous units present but he was quite certain that there were at least these two types in the algae.

WATER SOLUBLE POLYSACCHARIDE OF *Cladophora rupestris*

By I. S. Fisher and E. E. Percival (Scotland)

Read by E. E. Percival

Cladophora rupestris, a green seaweed with a high cellulose and protein content, was reported by H. Kylin[1] in 1944 to give a slimy water-soluble material which contained galactose and which was an ethereal sulphate of calcium and magnesium. As no other work has been published on the water-soluble polysaccharide of this weed it appeared highly desirable to investigate it further.

We are indebted to the Scottish Institute of Seaweed Research for the collection of the weed in November, 1953, from the East Coast of Scotland near Dunbar, between high and low tide. The weed was first freed from extraneous matter by washing with cold running water, and, after removal of some of the colouring matter by several treatments with cold alcohol, the polysaccharide was extracted with boiling water or with dilute hydrochloric acid (pH 3–4) at 70° C. Concentration of the extracts, under reduced pressure at 45° C, gave a viscous solution from which the polysaccharide was isolated, both by freeze-drying and by precipitation in alcohol, in an overall yield from the dried weed of 11·4%. This material had a nitrogen content of 3·0% which represents a protein content of 19·5%, and numerous attempts were made to purify it[2–11]. The most satisfactory and economical method was partial precipitation of the protein with 4% trichloroacetic acid, followed by fractional precipitation of the polysaccharide with alcohol. After removal of impure fractions with 50 and 70% alcohol, a material was precipitated with 90% alcohol, in approximately 5% yield by weight from the dried weed with $N = 1{\cdot}2\%$. All subsequent work was carried out on this material, hereinafter called polysaccharide A. It was an off-white powder, $[a]_D^{20} + 69°$, it was non-reducing and contained no ketose, amino sugar or uronic acid residue. It had OMe = 0; $CH_3CO = 0$, Ash, 13·7%, ash (as SO_4'') 16·7%, which had the composition SO_4 62·0%, Ca^{++} 22·1,

Fe^{+++} 4·4, Na^{+} 3·7, K^{+} 0·4, and an insoluble residue ($As_2O_3 + SiO_2$) 6·1%. While cations such as Ca^{++} could be precipitated, no SO_4'' could be detected in solutions of the polysaccharide until after hydrolysis. Furthermore the sulphate content of polysaccharide A was 19·6%, and that calculated from the ash 9·9%, a ratio of 2 : 1 which is characteristic of polysaccharide ethereal sulphates where:

$$2RO \cdot SO_2 \cdot ONa \xrightarrow{\text{heat}} Na_2SO_4 + SO_3 + \text{products of combustion}$$
(R = sugar residue)

and this polysaccharide is therefore a sulphuric ester similar to the polygalactan sulphuric ester of *Chondrus crispus*[12].

Hydrolysis of polysaccharide A with sulphuric acid, followed by chromatographic analysis of the resulting syrup showed the presence of arabinose, galactose, xylose, rhamnose and glucose, and quantitative estimation by elution on paper, and estimation with periodate[13] and by the Nelson-Symogyi[14] method, showed these sugars to be present in the relative molecular proportions of approximately 16 : 12 : 4 : 2 : 1 respectively. Furthermore, every sample of the polysaccharide isolated by the different methods of purification always gave, on hydrolysis, the same five sugars, in what appeared to be the same proportions when examined on the paper chromatogram. Partial hydrolysis with oxalic acid in an atmosphere of carbon dioxide, and separation of the hydrolysate on a cellulose column, led to the isolation of all five sugars in a crystalline form, together with a small quantity of a syrupy disaccharide. The sugars were characterised by melting point, rotation and the isolation of known crystalline derivatives. The disaccharide fraction, after purification, gave a single spot R_f 0·13, R_G 0·17 in butanol-pyridine-benzene-water (5 : 3 : 1 : 3) and, after hydrolysis, gave spots corresponding to glucose and arabinose. The possibility that this disaccharide was an artefact was disproved by the hydrolysis of an artificial mixture of chromatographically pure specimens of the five sugars under the conditions used for the hydrolysis and neutralisation of the polysaccharide. No disaccharide or any other reversion product could be detected in the solution on subsequent paper chromatography.

The possibility that polysaccharide A was more than a single entity has also been investigated. Fractional precipitation of the polysaccharide and the acetylated polysaccharide (Acetyl = 20%)

gave the five sugars in the same relative intensities, on hydrolysis of each of the fractions and examination on the paper chromatogram. Dr. C. T. GREENWOOD of the Chemistry Department, Edinburgh, kindly undertook electrophoresis in a 1% solution of this polysaccharide in borate buffer pH 10. He obtained a Schlieren diagram with a symmetrical peak, indicating a monodispersed polymer.

Partial removal of the sulphate residues was achieved by heating with *N*-sodium hydroxide at 90°, in an atmosphere of nitrogen, for 8 hours. A degraded polysaccharide was isolated in 51% yield, with sulphate = 13·6, and nitrogen = 0·6%. Examination of the hydrolysate of this degraded material showed no obvious change in the relative proportions of the sugars present. This degraded polysaccharide gave a positive Selivanoff test for an anhydro-sugar residue on standing overnight. Similar tests on polysaccharide A and on a blank gave negative results.

After conversion to the sodium salt, periodate uptake during 36 hours was equal to 347 g polysaccharide A, for each gram molecule of periodate. Treatment for 48 hours with periodate gave an oxypolysaccharide in 85% yield, which contained the five sugars, arabinose, galactose, xylose, rhamnose and glucose in molecular proportions approximately equal to 32 : 7 : <1 : 6 : 3 respectively. Comparison with the proportions found in the original polysaccharide showed that a considerable reduction in the quantity of galactose and xylose had taken place, and that the majority of the units of these two sugars had been attacked by periodate. A pentose residue, unless it occurs at the end of a polysaccharide chain, has only two free hydroxyl groups. If these are contiguous, then the residue will be attacked by periodate, but if either of these hydroxyl groups carry a sulphate group, then the pentose will be immune to attack by periodate. It would appear, therefore, that both the galactose and the xylose residues have adjacent free hydroxyl groups, since they are attacked by periodate, and that the xylose units are either situated at the ends of chains, or are not esterified with sulphate. Assuming xylose to be free from sulphate, then calculation shows that one in every two of the remaining sugar units in polysaccharide A carry a sulphate residue.

Partial hydrolysis experiments have been carried out in an attempt to isolate and identify small portions of the polysaccharide molecule. Hydrolysis with 0·1*N*-oxalic acid at room temperature

for 5 weeks split off a small quantity of galactose only; 8 hours at 50° with the same acid in an atmosphere of carbon dioxide removed mainly galactose and xylose with a trace of arabinose and a disaccharide. 0·5*N*-Oxalic acid, at 100°, for 7 hours, under carbon dioxide gave a hydrolysate, which contained all five sugars together with the previously isolated disaccharide, and a residual degraded polysaccharide was isolated in 8–10% yield from this solution.

Simultaneous deacetylation and methylation of polysaccharide A has been carried out, and a methylated product isolated with a methoxyl content of 20%. A preliminary hydrolysis of this material indicated the presence of tetramethyl galactose.

Briefly then, the water soluble polysaccharide from Cladophora rupestris appears to be a single individual. It is a sulphated polysaccharide, and contains the five sugars, arabinose, galactose, xylose, rhamnose and glucose; the first two sugars accounting for over three-quarters of the molecule. The majority of the galactose and xylose residues are attacked by periodate and must therefore contain two contiguous unsubstituted hydroxyl groups. Partial hydrolysis experiments preferentially remove galactose and xylose residues, indicating that these two sugars are situated near the ends of the polysaccharide chains. This is further supported for galactose by the identification of tetramethyl galactose, in the hydrolysate of the methylated polysaccharide.

Until more evidence is available from the methylation of the various fragments, and from the methylated whole polysaccharide, it is impossible to reach any further decision concerning the fine structure of this polymer, which in conclusion I would suggest might be named Cladophoran.

REFERENCES

1. Kylin, *Kgl. Fysiograf Sällskap. Lund. Forh.*, **14**, 221 (1944).
2. Hough Jones and Wadmann, *J.*, 3397 (1952).
3. Heuser, *J. Prakt. Chim.*, **104**, 261 (1922).
4. Percival, E. G. V. and Ross, *J.*, 717 (1950).
5. Doak, *N. Z. J. Sci. Tech.*, **31**, 2B, 90 (1939).
6. Engstrøm and Jakus, *Nature*, **161**, 168 (1948).
7. Cohn *et al.*, *J. Amer. Chem. Soc.*, **62**, 3386 (1940).
8. Hagedorn and Jensen, *Biochem. Z.*, **135**, 46 (1923).
9. Arni and E. G. V. Percival, *J.*, 1826 (1951).

10. Sevag, Lackmann and Smollens, *J. Biol. Chem.*, **124**, 425 (1938).
11. Stumpf, *J. Biol. Chem.*, 176 (1948).
12. Buchanan, Percival and Percival, *J.*, 51 (1943).
13. Hirst and Jones, *J.*, 1659 (1949).
14. Nelson, *Biochem. J.*, **53**, 375 (1944).

DISCUSSION

Dr. Young, Canada, stated that there was no question of the identity of the isolated sugar. In his experience, at least in the case of *Chondrus*, proteins are very difficult to dissolve. He asked Dr. Percival if the nitrogen might be in the form of amino acids or simple polypeptides, and if she had tried to remove the nitrogen by dialysis. Secondly, he was not convinced that the physical approach to homogeneity established it entirely since electrophoretic techniques are not quite satisfactory.

Dr. Percival answered that there might possibly be more than one polysaccharide. She had tried dialysis. Each fraction of the polysaccharide was dialysed for a week after treatment with trichloroacetic acid. She was not completely convinced from the physical evidence that the polysaccharide was homogeneous. All the methods she had tried up to the present had failed to show any separation whatsoever.

Mr. Jensen, Norway, asked Dr. Percival if she had determined the viscosity of the polysaccharide in solution, to which she answered that no viscosity measurements had been carried out.

Dr. Woodward, Scotland, was interested in the gelling properties of the polysaccharide and Dr. Percival stated that they got an increased viscosity by concentration, but the polysaccharide did not gel to the same extent as agar. She added that it had been suggested to her that the presence of anhydro sugar units had something to do with the gellifying ability but that they had failed to obtain any positive evidence for the presence of anhydro sugar units in the polysaccharide they had isolated.

SEASONAL VARIATION IN CHEMICAL COMPOSITION OF *Laminaria digitata* FROM DIFFERENT PARTS OF THE NORWEGIAN COAST

By A. Haug and A. Jensen (Norway)

Read by A. Haug

In our study of the chemical composition of Norwegian seaweeds we have investigated the seasonal variation of the main components of some of our more important seaweed species from different parts of the Norwegian coast. We shall report here some observations on the seasonal variation of *Laminaria digitata* from northern and southern parts of the coast.

Samples were collected each month from Reine during the years

Fig. 1.

1950–53, and from Vardø, Ingøy and Espevær during the year 1952. As shown in Fig. 1, Vardo, Ingøy and Reine are located in the northern part of Norway, while Espevær is on the southern part of the coast. Fig. 2 shows the content of ash, alginic acid,

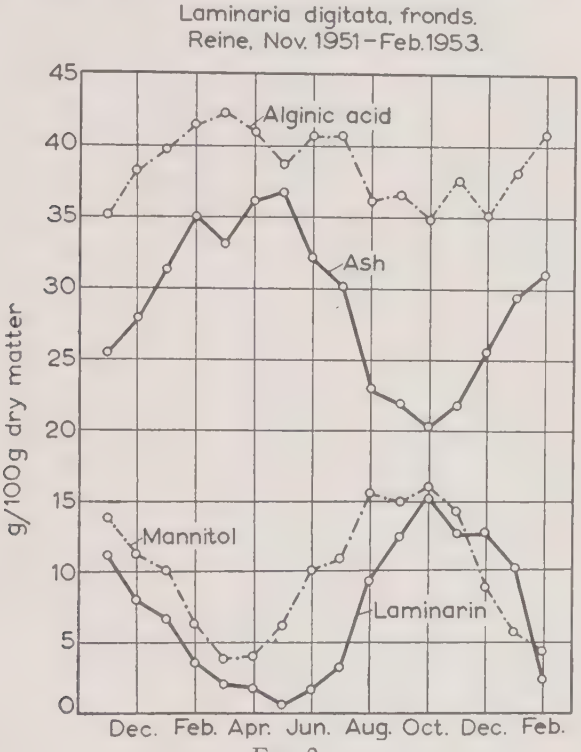


Fig. 2.

mannitol, and laminarin in *Laminaria digitata* fronds from Reine 1952. Apart from some minor differences, the seasonal variation of these compounds in the samples from Vardø and Ingøy followed the same general pattern, with one maximum and one minimum throughout the year.

Fig. 3 shows the composition of *L. digitata* fronds from Espevær. The laminarin content decreased rapidly from July to August and reached a second maximum in October. The ash content showed a maximum in August corresponding to the minimum in laminarin content. Fig. 4 demonstrates the difference in the seasonal variation of ash and laminarin in the samples from Vardø and Espevær.

Black has reported a similar drop in the laminarin content in the summer in *L. digitata* from two different localities in Scotland.

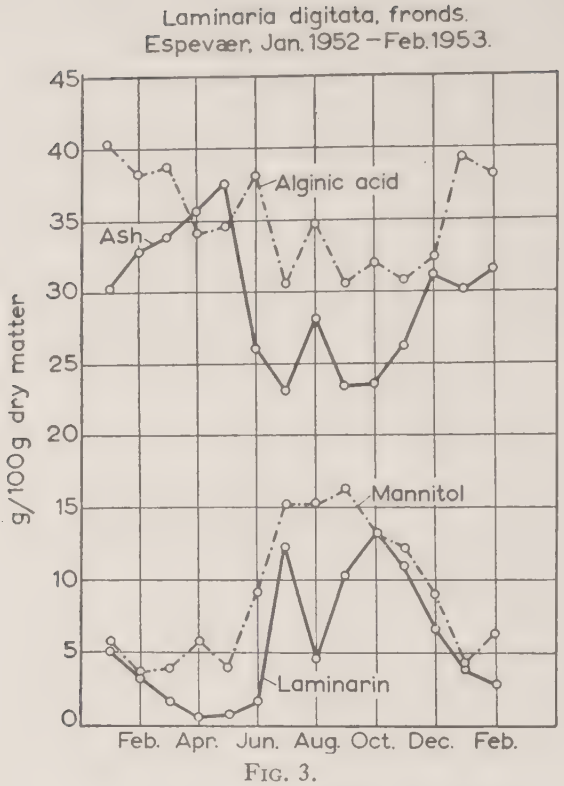

Laminaria digitata, fronds.
Espevær, Jan. 1952 – Feb. 1953.
g/100g dry matter
45
40
35
30
25
20
15
10
5
0
Alginic acid
Ash
Mannitol
Laminarin
Feb. Apr. Jun. Aug. Oct. Dec. Feb.

FIG. 3.

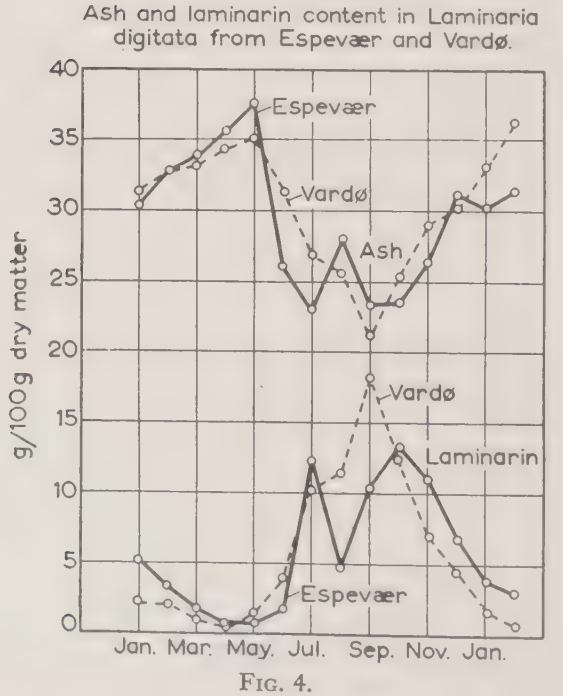

Ash and laminarin content in Laminaria digitata from Espevær and Vardø.
g/100g dry matter
40
35
30
25
20
15
10
5
0
Espevær
Vardø
Ash
Vardø
Laminarin
Espevær
Jan. Mar. May. Jul. Sep. Nov. Jan.

FIG. 4.

Fig. 5 shows the ash and laminarin content in *Laminaria digitata* from Espevær 1952 and Loch Melfort 1945. The curves agree fairly well. The main difference is the laminarin content in October, which is considerably higher in the plants from Scotland. BLACK has investigated the seasonal variation of the composition of *L. digitata* for four years. In the samples of the sea loch locality

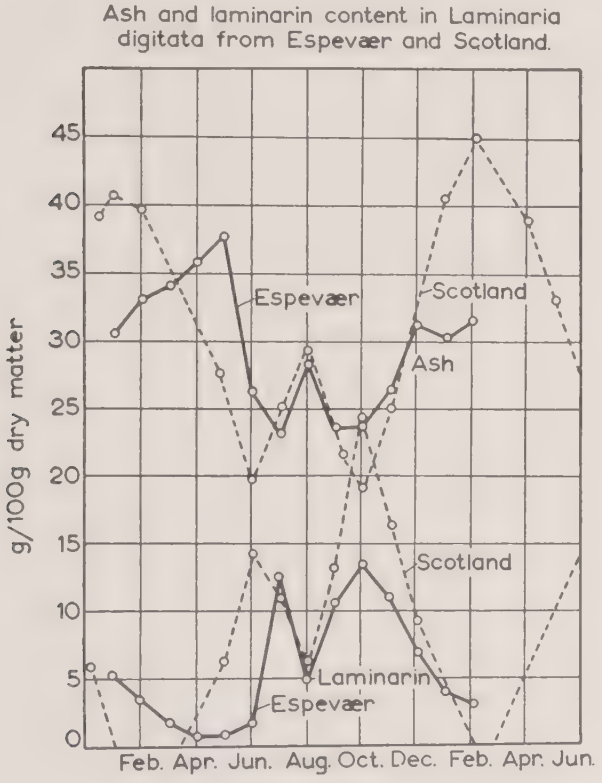


FIG. 5.

(Loch Melfort) he observed the laminarin drop in summer in the years, 1945, 1946 and 1948. In 1947 no minimum in the laminarin in summer was observed. In the samples from the open sea locality (Atlantic Bridge) the laminarin drop was observed in the years 1945 and 1947. BLACK also reported a corresponding minimum in the mannitol content in summer. No such minimum has been observed during our investigation.

In one of the discussions at the First International Seaweed Symposium, DILLON mentioned that in his investigations of the

laminarin content of *L. digitata* from Ireland he had observed a sudden drop in the laminarin content during July and August. In an investigation of *L. digitata* from the southwestern part of the Norwegian coast, LUNDE and collaborators observed a lower laminarin content in September than in August and October. LUNDE did not consider this minimum to be significant.

In the two latter investigations the size of the samples is not reported. In the investigations of BLACK, each sample consisted of two plants. The individual variation of the chemical composition of *L. digitata* plants, collected at the same time and from the same limited area on the shore, is shown by BAARDSETH and HAUG to be considerable. In 55 plants collected at Reine in August 1951 the ash content varied from 15 to 33% of the dry matter, with a standard deviation of 3·9%. This variation makes it obvious that curves representing the seasonal variation may become quite misleading if the investigation is based on samples consisting of only a few specimens.

Our samples consisted of at least 50 plants. Let us consider the ash content in the Espevær samples in July and August. In July the ash content was 23% of the dry matter and in August 28%. If we assume that the standard deviation of the ash content is the same in this population as in the 55 plants from Reine, i.e. 3·9%, we find a standard deviation of the difference between two samples, each consisting of 50 plants, to be 0·8%. The observed difference between the July and August samples is 5%, which is considerably more than three times this standard deviation. It is therefore very unlikely that the increase in ash content from July to August may be due to individual variation. As an increase in ash content usually corresponds to a decrease in the laminarin content, it can be concluded that the observed laminarin drop in August in the Espevær samples can not be explained as a result of individual variation.

The possibility remains that botanical heterogeneity may be the cause of the laminarin drop. In outer appearance, all plants in the Espevær samples were typical *L. digitata* of the narrow-leaved form. About 200 *L. digitata* plants from Espevær have been closely examined and it is found that two of these plants could be stained dark-red with tetrazotated benzidin. In no other case has it been observed that *L. digitata* plants could be stained in the same way by this reagent. In outer appearance these plants could not be dis-

tinguished from ordinary *L. digitata* plants, but an anatomical examination seemed to indicate that there might be some difference between these plants and ordinary *L. digitata*. This point will be further investigated.

The possibility of botanical heterogeneity in the Espevær samples can therefore not be excluded. However, it does not seem reasonable that this can be the explanation of the observed laminarin drop, considering the good agreement with the observations of BLACK, DILLON and LUNDE. It seems, therefore, reasonable to conclude that a drop in the laminarin content in summer may occur in *L. digitata* plants. The drop in the laminarin content in summer has not been observed in samples from the northern part of Norway. BLACK, DILLON and LUNDE collected their samples from localities south of Espevær. It is therefore probable that the latitude of growth may be of importance.

DISCUSSION

Dr. WOODWARD stated that the detailed investigation of the variation in laminarin content was important. His group had investigated *Macrocystis pyrifera* and *M. integrefolia* from Canada, South Africa and Australia. They had in no case been able to find any laminarin. Dr. WOODWARD asked Mr. HAUG if he had any idea of the distribution of the age groups in his samples.

Mr. HAUG could only say that the smallest plants were omitted. No other studies of the age distribution had been done.

Dr. SØRENSEN pointed out that the maximum content of laminarin varied considerably from year to year indicating that this compound is an excess factor to a certain extent.

Mr. FLOOD wanted to know from Mr. HAUG whether samples were always taken from the same population or haphazardly from a certain area of the coast.

Mr. HAUG answered that the plants were taken from what was thought to be the same population.

Mr. FLOOD wondered to what extent the population had been changed by taking away 50 plants every month over a long period.

Mr. HAUG was of the opinion that the population did not change much as a result of the sampling, which lasted for a little more than one year. To a question from Dr. WOODWARD about the depth from which the samples were taken, Mr. HAUG replied that the plants were collected by hand from the shore at low tide.

DR. SØRENSEN said that in Norway this is approximately 1 metre below the mean tide level.

CAROTENE CONTENT OF SOME NORWEGIAN SEAWEEDS, AND OBSERVATIONS ON THE BREAKDOWN OF CAROTENE IN SEAWEEDS AND SEAWEED MEAL

By A. Haug and B. Larsen (Norway)

INTRODUCTION

The carotene content of seaweeds has some importance when seaweed is used as stock food, on account of the vitamin A value of carotene. Several authors have investigated the carotenes and the carotenoids of algae, and in some cases quantitative determinations have been carried out. None of the carotenoids of algae should be expected to act as provitamin A. The only provitamin A in algae should therefore be the carotenes. In brown algae only β-carotene has been found[1]. Some red algae contain both α- and β-carotene[1].

Table 1 shows some of the quantitative determinations of carotene made by Seybold and Egle[2], and Owen[3]. The results differ

Table 1. Some Earlier Observations on the Carotene Content of Seaweeds

Species	Carotene content, mg per 1000 g dry matter. Seybold and Egle, Heligoland, Jul.–Aug.	Owen, Scotland, Sept.	Jan.
Fucus serratus	160		
Fucus vesiculosus	140	(58)	53 (35)
Ascophyllum nodosum	68	(25)	16 (24)
Ascophyllum nodosum, yellow	35		
Pelvetia canaliculata		(38)	38 (44)
Rhodymenia palmata	249–285	(115)	68 (37)
Gigartina stellata		(4)	
Chondrus crispus	93–167		

The figures in the brackets refer to determinations on dry material.

markedly from each other. It therefore seemed necessary to carry out further investigations on the carotene content of seaweeds. In

this work we have determined the carotene content of the more common *Fucaceae*, which are or may be used as stock food, and we have also carried out some investigations on the red algae *Rhodymenia palmata* (L.) Grev. The influence of the drying methods on the carotene content of seaweed meal has been investigated in some detail. All samples have been gathered in the Trondheimsfjord.

METHOD OF ANALYSIS

The analysis was carried out in the following way[4]: When a dry sample was analysed, a small amount of water was added. The sample was then extracted thoroughly with acetone, the carotene extracted from the acetone-water solution with light petroleum, and finally separated from chlorophyll and carotenoids by chromatography on magnesium oxide. The amount of carotene was determined in the Beckman spectrophotometer at 436 mμ. Fresh material was extracted in a Waring Blendor with acetone and the analysis proceeded as for dry samples.

The difference between the fresh samples and samples dried for a few days at room temperature was found to be insignificant. This does not apply to *Rhodymenia*, which will be considered later in a special section. Routine determinations of the carotene content of the three species *Ascophyllum nodosum* (L.) Le Jol., *Fucus serratus* L. and *Fucus vesiculosus* L. may therefore safely be carried out on material dried at room temperature.

CAROTENE CONTENT

Table 2 shows that the carotene content increases from February

Table 2. Carotene Content of some Norwegian Seaweeds

Species	May–54	Feb.–55	Mar.–55	Apr.–55	May–55	June–55
Ascophyllum nodosum	78	50	46	43	58	61
Fucus serratus	205	105	114	135	121	174
Fucus vesiculosus	200	95	110	107	119	113
Fucus spiralis	110					
Pelvetia canaliculata	110					
Rhodymenia palmata	270(f)	197(f)	217(f)	282(b)	388(b)	372(b)

f = fresh samples
b = steam blanched samples

to June. The investigations will be carried on throughout a year. Our results agree fairly well with those of SEYBOLD and EGLE, but are considerably higher than those of OWEN. OWEN suggested that the difference in carotene content between his and SEYBOLD and EGLE's series of seaweeds might be due to the difference in latitude of growth. As OWEN's samples are taken at a latitude between Heligoland and the Trondheimsfjord this explanation seems improbable.

VARIOUS DRYING METHODS

Table 3 shows the effect of various drying methods on the carotene content of *Ascophyllum nodosum* and *Fucus serratus*. Open air

Table 3. Drying Experiments

The figures are given as percentage of the content in the sample dried at room temperature

Drying conditions	*Asc. nodosum*, May–55	*Fuc. serratus*, Apr.–55
Room temp. 6 days	100 (58)	100 (135)
Open air drying, 6 days — protected against rain	100	87
Open air drying, 6 days — exposed to rain	91	54
Artificial drying 50°C, 22 h.	91	90
Artificial drying 80°C, 3 h.	90	100

The figures in the brackets are given as mg carotene per 1000g dry matter

drying for 6 days or drying at temperatures up to 80° C led to no significant carotene breakdown in *Ascophyllum*. With the exception of the samples subjected to rain, no appreciable difference between the two species was observed. Rain during open air drying led to a considerable decrease in the carotene content of *Fucus serratus*, while no breakdown was observed in *Ascophyllum* when the open air drying did not exceed one week. Prolonged open air drying, however, led to a marked loss of carotene in both species.

Fig. 1 shows the breakdown of carotene in *Ascophyllum* dried in open air. A similar experiment with other species is shown in Fig. 2. We want to point out the remarkable stability of carotene in *Pelvetia canaliculata* (L.) Dcne. et Thur. and the extraordinary rapid breakdown in *Rhodymenia palmata*.

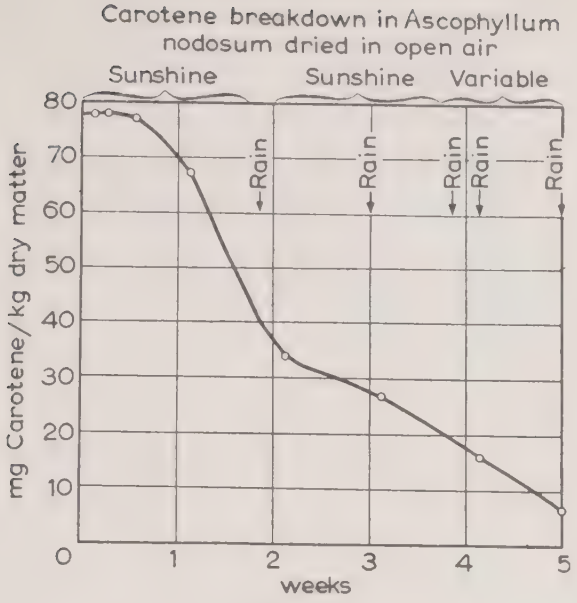

Carotene breakdown in Ascophyllum nodosum dried in open air
Sunshine
Sunshine
Variable
Rain
Rain
Rain
Rain
Rain
mg Carotene/kg dry matter
80
70
60
50
40
30
20
10
0
1
2
3
4
5
weeks

FIG. 1.

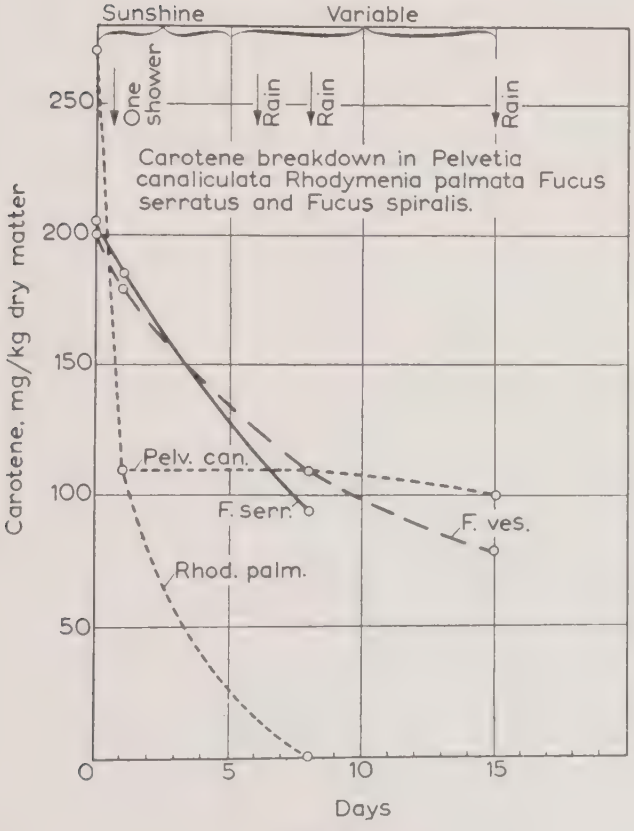

Sunshine
Variable
One shower
Rain
Rain
Rain
Carotene breakdown in Pelvetia canaliculata Rhodymenia palmata Fucus serratus and Fucus spiralis.
Carotene, mg/kg dry matter
250
200
150
100
50
0
Pelv. can.
F. serr.
F. ves.
Rhod. palm.
5
10
15
Days

FIG. 2.

STORAGE OF SEAWEED MEAL

Fig. 3 shows the breakdown of carotene in seaweed meal stored in airtight tins. All samples are previously dried at room tempera-

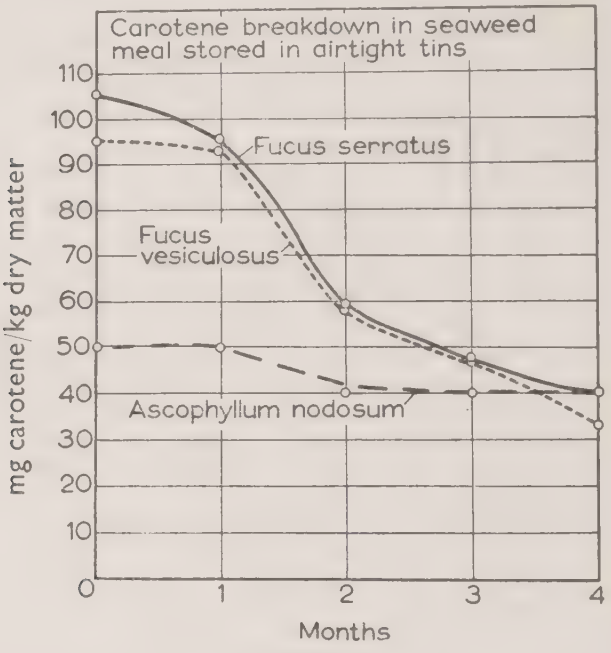


Fig. 3.

ture. Four months storage led to only a slight decrease in the carotene content of *Ascophyllum nodosum*, while more than half of the carotene was lost in *Fucus serratus* and *vesiculosus*.

Conclusions on the drying experiments:

If we want a seaweed meal with a high carotene content, prolonged open air drying must be avoided. The seaweed should be protected against rain. The best results will probably be obtained with artificial drying. Temperatures up to 80° C were tried and found to lead to no carotene loss in either species.

Even when care was taken to avoid carotene breakdown during drying, about 50% of the carotene in *Fucus serratus* and *Fucus vesiculosus* was lost when the meal was stored for 3–4 months. In artificially dried *Ascophyllum* meal, however, the carotene loss during four months storage was only approximately 20%.

BIOLOGICAL EXPERIMENTS

Experiments were carried out by the firm of Collett & Co. to determine the biological vitamin A value of two different samples of *Ascophyllum* meal and one sample of *Fucus serratus* meal. The animals used in the experiments were rats. In all cases the observed biological vitamin A value corresponded to between 80 and 100% of the observed carotene content of the samples. It may thus be concluded that the Vitamin A value in seaweed meal is due only to the carotene content, and may be estimated by a determination of the carotene.

SOME OBSERVATIONS ON THE CAROTENE BREAKDOWN IN *Rhodymenia palmata*

The very rapid breakdown of carotene in *Rhodymenia palmata* made this alga a convenient object for further studies of the carotene breakdown. Contrary to the observations of HEILBRON[5], we found that both α- and β-carotene were present in this alga in approximately equal amounts. Drying experiments showed that UV-light and temperature up to 100° C did not increase the carotene breakdown compared with samples dried at room temperature in diffuse light. Rain, on the other hand, led to a very rapid breakdown of the carotene. The highest carotene content was observed in blanched samples.

These experiments seemed to indicate that the breakdown is of enzymatic nature. The fresh algae were ground in a mortar with solid carbon dixoide and quartz sand. When set aside at room temperature for 20 hours before extraction with acetone the carotene was completely destroyed. The breakdown was almost completely inhibited by 0·5% potassium cyanide and 0·5% sodium sulphide, while the same amount of mercuric chloride only slightly inhibited the reaction and 0·5% sodium fluoride had no effect.

It seems to be safe to conclude that an enzymatic reaction must be responsible for the rapid carotene breakdown in *Rhodymenia palmata*.

REFERENCES

1. GOODWIN, T. W., *The Comparative Biochemistry of the Carotenoids*, Chapman and Hall, London 1952.
2. SEYBOLD, A. und EGLE, K., *Jahrb. wiss. Botan.*, **86**, 50 (1938).
3. OWEN, E. C., *J. Sci. Food Agric.*, **5**, 449 (1954).

4. Ass. of Vitamin Chemist, Inc., *Methods of Vitamin Assay*, New York 1947.
5. HEILBRON, I. M. and PHIPERS, R. F., *Biochem. J.*, **29**, 1369 (1935).

DISCUSSION[1]

Dr. YOUNG asked Mr. HAUG if he considered *Rhodymenia palmata* an exceptional case among the rockweeds. Mr. HAUG answered that the same acceleration of the breakdown of β-carotene caused by rain was observed in *Fucus serratus* too.

Dr. YOUNG then wanted to know if artificial drying at higher temperatures would give better protection of the carotene and this was verified by Mr. HAUG. Dr. YOUNG asked Mr. HAUG whether the breakdown might be related to the fat content. Mr. HAUG answered that as lipoxidase is indirectly responsible for the breakdown of carotene in higher plants, this might also be the case in certain algae. Dr. FREUDENTHAL pointed out the difference observed between biological tests and chemical determinations and wanted to know if Mr. HAUG had carried out any biological experiments to determine the vitamin content.

Dr. SØRENSEN, Norway, stressed that the Norwegian Institute of Seaweed Research had taken great interest in the possible presence of any vitamin A active substances other than the well known provitamin A (β-carotene). Very reliable biological tests had been carried out and activities corresponding to 80 to 100% of the β-carotene were found. This completely ruled out the possibility of other vitamin A active substances being present in these algae (*Ascophyllum nodosum* and *Fucus serratus*). Dr. SØRENSEN said he found it very important to determine what vitamins are present in algae, and pointed out the discrepancies frequently observed between biological and chemical tests. He stressed the absolute necessity of common definitions of units and methods in order to overcome these difficulties. Concerning the vitamin A content, the values obtained by Dr. FREUDENTHAL agreed very well with the values usually obtained for a commercial seaweed meal, so at this point no discrepancies seemed to occur. Dr. SØRENSEN could thus see no reason to *postulate* the presence of any active substances in addition to β-carotene. Then turning to vitamin D, Dr. SØRENSEN doubted that Dr. FREUDENTHAL's experiments were properly set up, because an experiment with vitamin D has to be based on a diet adequate in vitamin A. This was not the case in Dr. FREUDENTHAL's experiments as his concept of vitamins mixed together the A, D, and E vitamins. In this respect Dr. FREUDENTHAL's experiments were not concordant with the hundreds of other biological experiments carried out all over the world, and his results were not comparable with those obtained according to the international standards.

[1] Refers also to the paper by FREUDENTHAL, p. 62

SOME CONSTITUENTS OF *Porphyridium cruentum*

By F. T. Haxo and Colm O'h Cocha (U.S.A.)

Read by F. T. Haxo

ABSTRACT

Porphyridium cruentum is a unicellular red alga which grows on soil and damp walls and has also been reported from the sea. The cells used in these experiments were grown in mineral-enriched seawater. The culture medium becomes viscous due to dissolution of extra-cellular carbohydrates; very old cultures are semi-solid. It may be that such extra-cellular mucilages will find economic application.

One litre of medium from a pure culture of *Porphyridium* yielded 0·4 g of purified fibrous, gel-forming material, which was hydrolysed with dilute acid (the number of polysaccharides present was not determined). The following compounds were found in the hydrolysate: glucose, galactose, and xylose (identified by paper chromatography) and uronic acid (positive naptho-resorcinol test). The driest sample obtained contained ca.10% ethereally bound SO_4. The *Porphyridium* mucilage thus appears to be, at least in part, a sulphated polysaccharide.

The colouring matter of *Porphyridium* has been examined, employing conventional chromatographic adsorption analysis for the fat-soluble components and separation on tri-calcium phosphate gel for the chromoproteins. Pigments included in the former group are chlorophyll a (chlorophyll d was not detected), β-carotene, zeaxanthin, and small amounts of a xanthophyll tentatively identified as lutein.

The bilichromoprotein mixture contains as its major component a phycoerythrin absorbing maximally at 545 mμ, and small amounts of two phycocyanins, one with maxima at 554 and 617 mμ, the other with a single maximum at 650 mμ. The phycoerythrin from *Porphyridium* differs spectrally from both the R- and C-varieties of this pigment, yet all of the phycoerythrins examined

yielded on short anaerobic acid hydrolysis a similar chloroform-soluble bile pigment displaying a maximum at 577 mμ. The spectral differences observed between phycoerythrin varieties would seem, therefore, to be due to different proteins or to protein-chromophore linkages.

DISCUSSION

Mrs. Kylin mentioned that *Porphyridium* was very easily cultivated and that it seemed to produce an antibiotic substance since no diatoms occurred in the cultures.

Dr. Haxo agreed that *Porphyridium* grows well enough for experimental purposes, but it was not easy to cultivate it on a large scale as *Chlorella* or *Scenedesmus*.

Dr. Bergmann wanted to know if Dr. Haxo had any rough idea of what the fat content might be, to which Dr. Haxo said that this point had not been investigated. Dr. Bergmann stressed the importance of obtaining such data for all plants that could be cultivated.

Dr. Young asked how Dr. Haxo had extracted the proteins from the whole cell, and what proportion of the total nitrogen in the plant was made up by the chromoproteins. He also wanted to know if Dr. Haxo felt reasonably confident that the chromophore had a pyrrole structure.

Dr. Haxo replied that the extractibility differed from species to species and that he had succeeded only with great difficulty in getting out the proteins from certain red algae like *Porphyra*. In the case of *Porphyridium* extraction following freezing, thawing and quartz grinding gave good results. The linear tetrapyrrolic nature of the phycobilin chromophore seems well established by earlier investigation. The identity of the side groupings is a point which ought to be investigated further. Dr. Haxo had no information to give on the proportion of chromoprotein to total nitrogen of the algae.

POSTMORTAL CHANGES IN THE CAROTENOIDS OF *Fucus vesiculosus*

By S. Liaaen and N. A. Sørensen (Norway)

Read by N. A. Sørensen

Since the classical investigations of Willstätter and Page the chloroplast pigments of the algae have been known for their great instability. The chlorophylls of brown algae can only be isolated from very fresh material, and the main carotenoid, fucoxanthin, will undergo chemical transformation if the algae are not dried rapidly at low temperature, and the extraction carried out in a few days. In 1935 Sir Ian Heilbron with R. F. Phipers[1] described the isolation of zeaxanthin from *Fucus vesiculosus* air-dried over a prolonged period. Fucoxanthin was absent in this material, and Heilbron and Phipers assumed that the isolated zeaxanthin had arisen through a postmortal change of the fucoxanthin. Investigations carried out later by H. Kylin[2] and by H. Strain[3] have not been able to confirm these transformations.

As our knowledge about the constitution of fucoxanthin is very limited, it would obviously have been very useful to get confirmation of a postmortal change of fucoxanthin into zeaxanthin, and for this reason Miss Synnøve Liaaen has carried out a re-investigation.

There is no general agreement about the carotenoids present in living *Fucus vesiculosus*; the re-investigation gave for our winter samples the following figures:

	Present investigation	Willstätter & Page	Seybold & Egle[4]
β-Carotene (with stereo-isomers)	110	267	144
Fucoxanthin	600	507	504
Neo-fucoxanthin	120		
Violaxanthin	180	261	144
Lutein	8		
Neoxanthin	traces		
Zeaxanthin	0		

All values recalculated to mg/kg dry algae.

WILLSTÄTTER and PAGE described the occurrence of some 261 mg/kg 'xanthophyll', the composition of this fraction being unknown. KYLIN found both violaxanthin, lutein and zeaxanthin. HEILBRON and PHIPERS do not mention any xanthophylls; STRAIN and his collaborators found violaxanthin, but not lutein. Neither WILLSTÄTTER nor STRAIN investigated just *Fucus vesiculosus*, but other *Fucus* species.)

Miss LIAAEN found that there were no visible traces of zeaxanthin in living *Fucus vesiculosus*. The small amount of lutein is not directly visible on a chromatogram on sucrose since it follows some decomposition products of chlorophyll. It is best taken out together with this chlorophyll-fraction and purified through saponification. These traces of lutein cannot be demonstrated in the presence of an excess of zeaxanthin. Special experiments revealed that none of the xanthophylls does occur esterified.

Next the composition of the carotenoids of carefully dried *Fucus vesiculosus* was investigated. No zeaxanthin was found, either immediately, or after 15 days at room temperature (cf. Table 1). There was a marked loss in β-carotene and in violaxanthin, whereas fucoxanthin remained unchanged. This observation is in agreement with the experimental procedure recommended by P. KARRER and his collaborators in 1931[5] for the preparative isolation of fucoxanthin.

Table 1

Carotenoid	Fresh algae	Dried in a vacuum 15 days	Stored in H_2S 3 days	Stored in water vapour 15 mm, 1 day	Stored in seawater sterile 3 days	Stored in seawater unsterile 8 days
β-Carotene	110	71	106	109	107	126
Fucoxanthin	720	678	749	738	778	800
Violaxanthin	180	77	38	44	50	19
Zeaxanthin	0	78	146	144	141	150
Lutein	8	—	—	—	—	—

The dried *Fucus vesiculosus* investigated by Sir IAN had been 'air-dried over a prolonged period', and Miss LIAAEN then carried out a series of experiments in order to see if experimental conditions could be found under which zeaxanthin is synthesised. And I am glad to be able to state that Miss LIAAEN found a lot of them leading to a smooth synthesis of zeaxanthin, as will be seen from Table 1 where the stereoisomeric fucoxanthins are taken together.

Since drying in a vacuum gives rise, curiously enough, to an appreciable amount of zeaxanthin, the idea presented itself that the process was favoured by the absence of oxygen. Columns 3 and 4 seem to confirm this hypothesis; in neither case does any drying of the algae take place. It was then tempting to leave the algae in seawater. Of two parallel samples, one was kept sterile; in both an appreciable synthesis of zeaxanthin took place.

These re-investigations confirm the statement of Sir IAN and his collaborators that zeaxanthin may arise as a postmortal product in *Fucus vesiculosus*. But at the same time the reinvestigation established that the fucoxanthin and the β-carotene in the algae remain unchanged during this synthesis, whereas violaxanthin is lost in an amount corresponding exactly to the zeaxanthin synthesised. This process has therefore no bearing on the constitution of fucoxanthin. Since violaxanthin, according to the brilliant investigations of PAUL KARRER and ERNST JUCKER[6], is the di-epoxyde of zeaxanthin, the post-mortal process observed is chemically the very simple one of a splitting-off of two epoxydic oxygens. KARRER discovered that the 5-6-epoxydes of the carotenoids were much more labile than most other known epoxides, in part loosing their oxygen by treatment with weak acids. Miss LIAAEN has tested the pH in the dying *Fucus vesiculosus* which, however, is neutral to weakly acid (pH = 6·2).

For calibration of the spectrophotometric determination of fucoxanthin, Miss LIAAEN had to prepare chromatographically pure fucoxanthin. Some of this pure fucoxanthin was used to obtain an infrared spectrum of fucoxanthin ($CHCl_3$-sol. and KBr-disk). Fig. 1 gives the carbonyl region for fucoxanthin, rhodoxanthin and astaxanthin. As will be seen, fucoxanthin must contain 2 different carbonyl functions. The band at 1660 cm^{-1} coincides with that of rhodoxanthin and astaxanthin and so undoubtedly belong to a keto-group conjugated with a long unsaturated chain. This keto-group is undoubtedly responsible for the colour change which fucoxanthin undergoes from a solution in petrol to a solution in alcohol. The second band at 1734 ± 5 cm^{-1} could belong to an isolated carbonyl group. Of the 6 oxygen atoms of fucoxanthin, two thus belong to two different types of carbonyl functions and the molecule must be nonsymmetric. As will be seen from Fig. 2, the tetraketo-carotenoid astacin has carbonyl

bands at much lower frequencies, 1626 and 1560 cm^{-1}, very close to those of the tropolones[7], positions which are undoubtedly characteristic of diosphenols conjugated with double bonds.

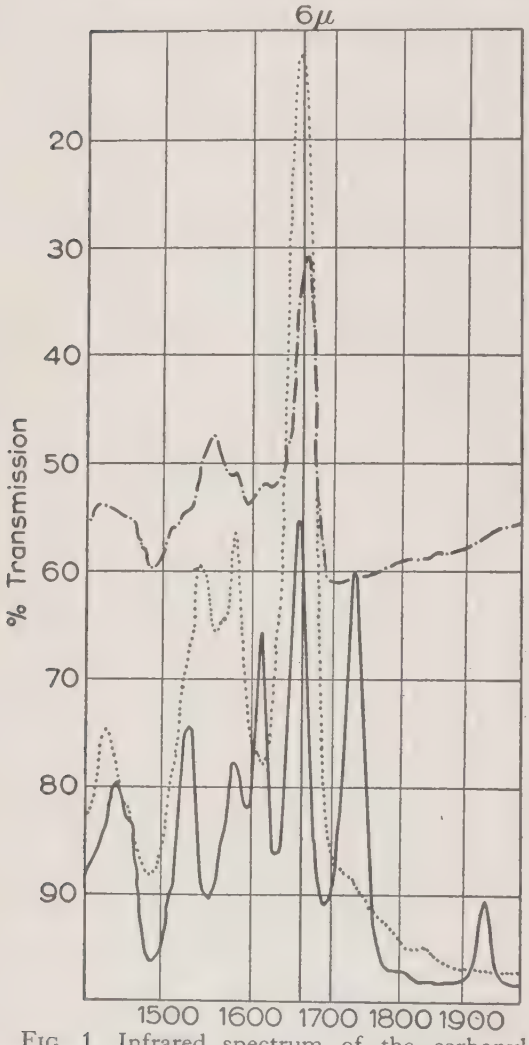


FIG. 1. Infrared spectrum of the carbonyl region of carotenoids in $CHCl_3$. ———— Fucoxanthin, ... Rhodoxanthin, . – . – . Astaxanthin, (the Astaxanthin curve is arbitrarily displaced 40% in transmission to avoid overlapping).

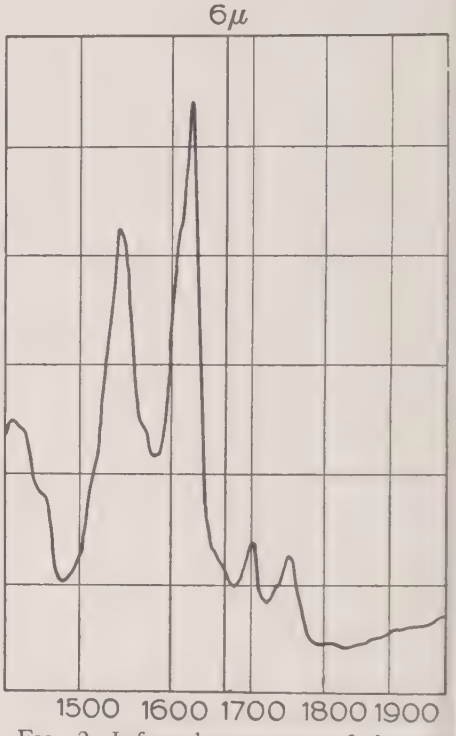


FIG. 2. Infrared spectrum of the carbonyl region of astacin.

The most unexpected band in the fucoxanthin spectrum is the peak of medium intensity at 1935 cm^{-1}. This position is characteristic for allene groupings —C=C=C—[8].

Fucoxanthin has long been known to be extremely alkali-sensitive. CELMER and SOLOMONS[9], and E. R. H. JONES *et al.*[10], have demonstrated that unsaturated carbonyl compounds containing an allenic group rearrange in as weak alkali as K_2CO_3 in methanol to a complicated mixture of enynic and polyenic compounds. Mrs. J. STENE SØRENSEN has carried out some preliminary investigations on the alkali isomerisation of fucoxanthin.

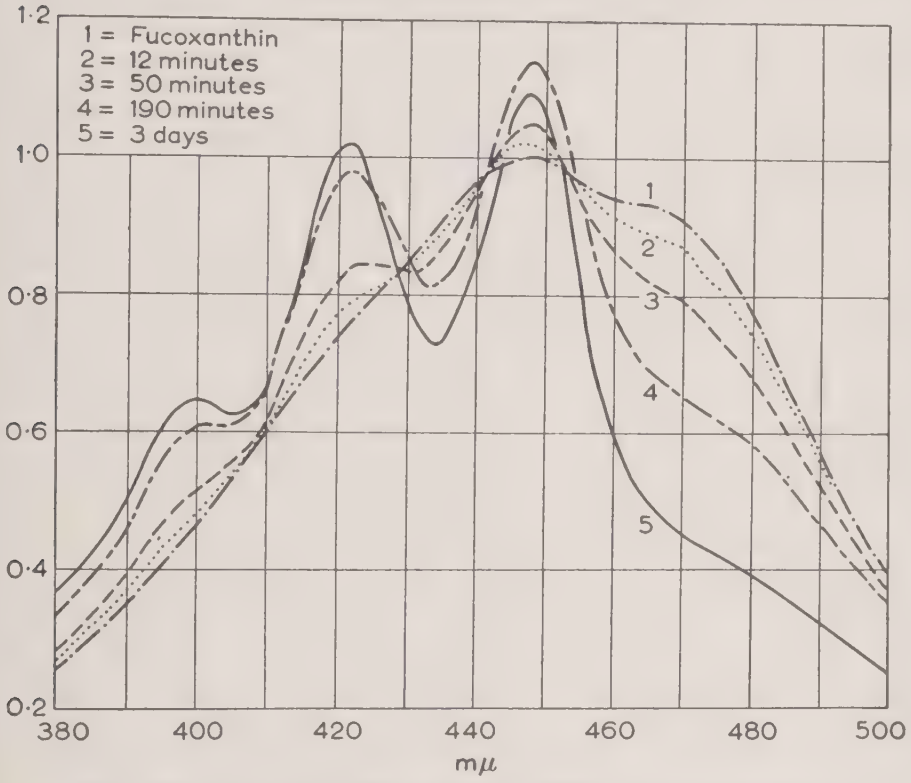


FIG. 3. Spectral change of fucoxanthin in 0·08% K_2CO_3 in methanol.

Fig. 3 shows the spectral change of fucoxanthin in 0·08% K_2CO_3 in methanol at room temperature. The bands move towards shorter wave-lengths and increase markedly in fine structure. Chromatography disclosed the formation of a complex mixture of new carotenoid pigments. The spectra of the 5 main bands are reproduced in Fig. 4.

Although three of the fractions still have a fucoxanthin like band, none of them represent unchanged fucoxanthin since infrared spectra, Fig. 5, showed that all had lost the conjugated keto-

peak at 1660 cm^{-1}. The band at 1730–1740 cm^{-1} is complex in the 4 alkali-isomers investigated. All four still show the peak at

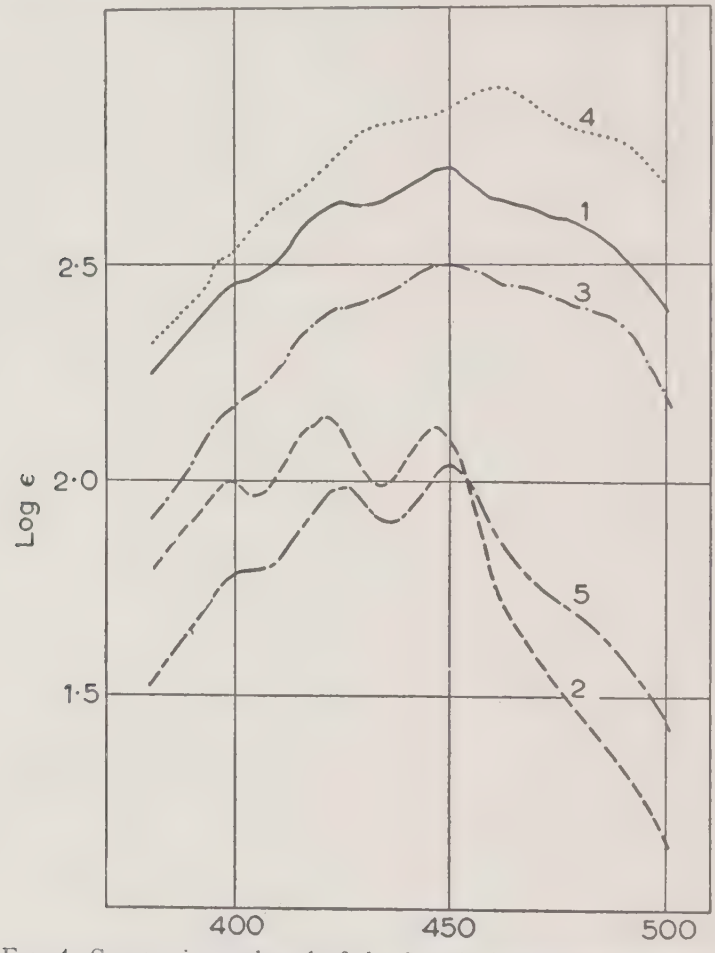


FIG. 4. Spectra in methanol of the 5 main isomerisation products of fucoxanthin in K_2CO_3-methanol. The numbering refers to the chromatographic zones on sucrose.

1935 cm^{-1}. Although all four were cautiously measured in the 2200 cm^{-1} region, no peak indicating a generation of acetylenic groupings could be found.

The fractions 2 and 5 which showed good fine structure have the first maximum at 446 and 449 mμ in methanol that is corresponding to a chain of 8 conjugated double bonds.

The allenic peak in fucoxanthin should give a valuable attack on

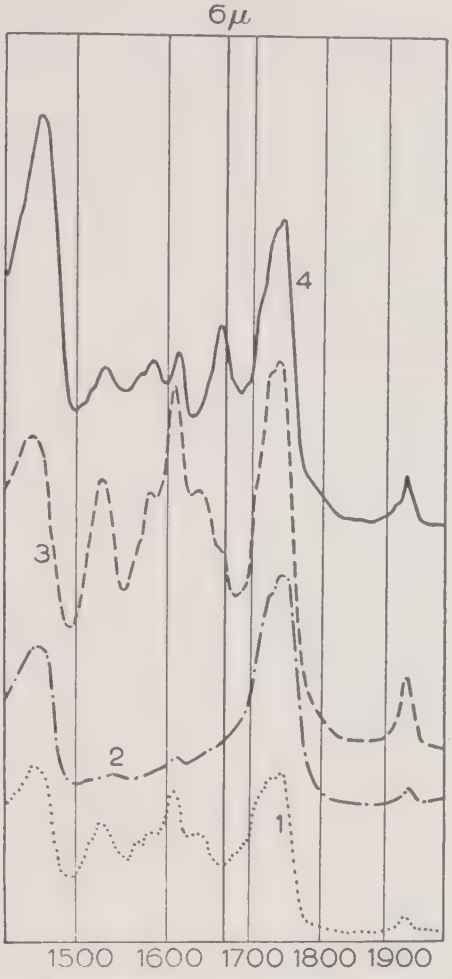


FIG. 5. Infrared spectra of the carbonyl region of four of the isomerisation products of fucoxanthin. Numbers correspond to fig. 4.

the constitutional problem of fucoxanthin, the last main carotenoid still lacking a formula.

REFERENCES

1. HEILBRON, I. M. and PHIPERS, R. F., *Biochem. J.*, 1369 (1935).
2. KYLIN, H., *Kungl. Fysiogr. Sällsk. Lund Forh.*, **9**, 211 (1939).
3. STRAIN, H. H., *Photosynthesis in Plants*, Iowa State Coll. Press, 1949.
4. SEYBOLD, A. and EGLE, K., *Jahrb. Wiss. Bot.*, **86**, 50 (1938).
5. KARRER, P., HELFENSTEIN, A., WEHRLI, H., PIEPER, B. and MORF, R., *Helv. Chim. Acta*, **14**, 628 (1931).

6. KARRER, P. and JUCKER, E., *Helv. Chim. Acta*, **28**, 300 (1945).
7. SCOTT, G. P. and TARBELL, D. S.: *J. Amer. Chem. Soc.*, **72**, 240 (1950); TARBELL, D. S., SCOTT, G. P. and KEMP, A. D.; *ibid.*, **72**, 379 (1950); AULIN-ERDTMAN, G. and THEORELL, H., *Acta Chem. Scand.*, **4**, 1490 (1950).
8. WOTIZ, J. H. and CELMER, W. D.: *J. Amer. Chem. Soc.*, **74**, 1860 (1952).
9. CELMER, W. D. and SOLOMONS, I. A.: *J. Amer. Chem. Soc.*, **75**, 1375 (1953).
10. JONES, E. R. H., WHITMAN, G. H. and WHITING, M. C.: *J. Chem. Soc.*, 3201 (1954).

DISCUSSION

Dr. FREUDENTHAL, Denmark, suggested that the additional vitamin activity observed by him had to be found in the fucoxanthin group of compounds. Only biological tests with pure fucoxanthin could conclusively prove or disprove this hypothesis. Dr. SØRENSEN agreed that only the biological tests would give a decisive answer, but at the same time he pointed out that these tests already had been carried out with fucoxanthin. As a result of repeated tests it was found that carotenoids with oxygen in both rings were entirely inactive. Dr. SØRENSEN also suggested that part of the activity observed by Dr. FREUDENTHAL in his experiments might be due to a relatively high content of tocopherols in the seaweed meal.

LOW-MOLECULAR WEIGHT CARBOHYDRATES IN BROWN AND RED ALGAE

By B. Lindberg (Sweden)

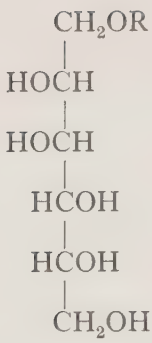
Starting from investigations of lichens being carried out at the Department, we became interested in the chemistry of algae, especially in low-molecular weight carbohydrates found in them, and we have during the last years investigated a number of marine algae. Previous investigations of these plants were rather incomplete, most efforts being concentrated upon the economically important polysaccharides, and the aim of our work was to try to find new compounds which might give a guide to the carbohydrate metabolism of these plants and, if possible, to try to find a basis for a chemical taxonomy. The main purpose has been realised to some extent, some new substances and some others, not previously found in algae, having been isolated. The second line of investigation, however, has been less successful. The brown algae all showed the same general pattern, while for the red algae the picture proved to be quite complicated, as already evident from the extensive work by Augier and his group[1].

In the following a summary of our results, put in relation to those of other investigations, is given.

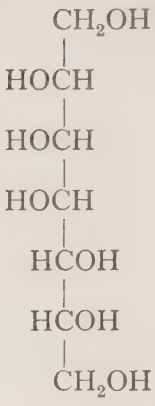
BROWN ALGAE

1. *Sugar alcohols*—D-mannitol (I) has been found in several brown algae and was isolated from all species investigated[2, 3, 4, 5]. This was the only sugar alcohol found, except in *Pelvetia canaliculata*[3] which also contained a high percentage of D-volemitol (II). This alga is found on the high-water line and is under water only for short periods. It is possible, therefore, that it produces this mixture of sugar alcohols in order to prevent crystallisation and to preserve its water during the dry periods. 1-D-mannitol acetate was isolated from *Fucus*[2, 5] and *Laminaria*[4] species, and the substance was also synthesised[6].

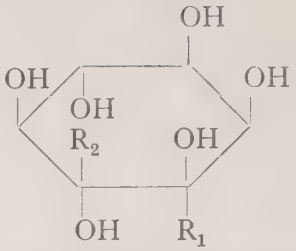
2. *Cyclitols*—A new cyclitol, laminitol, was found in *Laminaria*



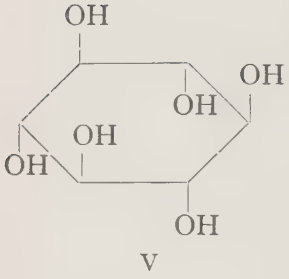
I R = H
IV R = β-glucose



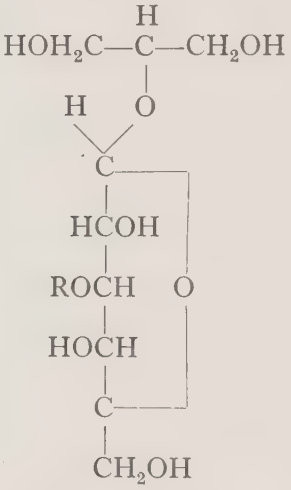
II



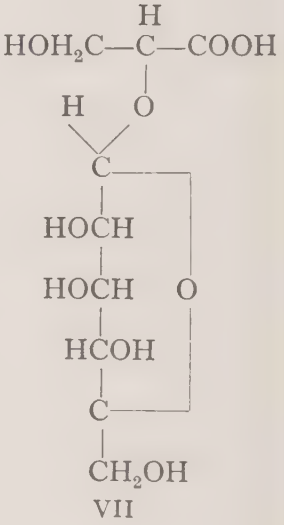
IIIa $R_1 = CH_3$, $R_2 = H$
IIIb $R_1 = H$, $R_2 = CH_3$



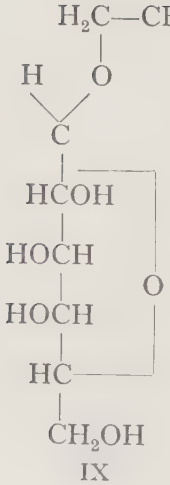
V



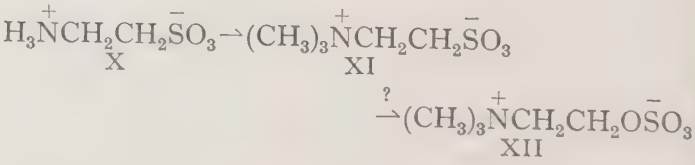
VI R = H
VIII R = α-mannose



VII



IX



X → XI → XII

cloustoni[7] and other brown algae[5]. It is a C-methyl inositol, and work on its configuration is in progress. It is optically active and there are indications that it has the meso-inositol configuration. The configuration is therefore probably that of III*a* or III*b* (or the corresponding antipodes) and for certain reasons we prefer that of III*a*.

3. *Glycosides*—All brown algae investigated[2, 3, 4, 5] contained 1-D-mannitol β-D-glucopyranoside (IV) and 1,6-D-mannitol di-(β-D-glucopyranoside). The monoglucoside was synthesised[8] and has recently been isolated by PEAT, WHELAN and LAWLEY[9] from partially hydrolysed laminarin. *Pelvetia canaliculata*[3] also contained the corresponding β-glucosides of D-volemitol, and as the two primary hydroxyl groups in volemitol are not identical, there are two monoglucosides, one of which (1 or 7)-D-volemitol β-D-glucoside, was isolated in a state of purity.

RED ALGAE

1. *Sugar alcohols*—Sorbitol and dulcitol were isolated from *Bostrychia scorpoides* by HAAS and HILL[10] and dulcitol from *Iridea laminaroides* by HASSID[11]. The occurrence of these substances in other red algae has not been reported and they were not found in other red algae investigated by us. A reinvestigation of *B. scorpoides* confirmed the observation of HAAS and HILL. For taxonomical reasons it is difficult to understand why *B. scorpoides* should differ from the other algae of the order *Ceramiales* and also why *Pelvetia canaliculata* should differ from the other brown algae. Both algae, however, live on the high-water line and it is possible that the hexitol mixture in *B. scorpoides* is produced for similar reasons to those discussed for the volemitol in *P. canaliculata*. Glycerol was found in all algae containing floridoside. D-mannitol, not previously isolated from red algae, was obtained from *Furcellaria fastigiata*[18], *Gigartina stellata*[19], and *Porphyra umbilicalis*[18], and from the last named D-volemitol was also isolated. The percentages of these substances, however, were so low that the possibility that they derive from impurities in the plant material is not excluded.

2. *Cyclitols*—Cyclitols have not previously been isolated from red algae. Meso-inositol was isolated from some algae in our investigations but is probably present in most plants. More interest-

ing is the isolation of scyllitol (V) and laminitol from *Porphyra umbilicalis*[18]. Scyllitol was not found in the other algae but there is evidence for the presence of laminitol in *Gelidium cartilagineum*[19]. The isolation of scyllitol and iso-floridoside (see below) from *P. umbilicalis* may be of taxonomical interest, as this alga belongs to a group, *Bangioideae*, distinguished from the main group, Florideae, in which these substances are not found.

3. *Glycosides*—A glycerol α-D-galactoside, floridoside, was first isolated by COLIN and GUEGUEN[12] and was proved to be 2-glycerol α-D-galactopyranoside (VI) by PUTMAN and HASSID[13]. A D-glyceric acid α-D-mannoside first isolated by COLIN and AUGIER[14], has now been proved by us, utilising the methylation procedure, to be a 2-D-glyceric acid α-D-mannopyranoside (VII)[18]. KAWAGUCHI, YAMADA and MIYAMA[15] have recently arrived at the same formula from a study of the lead tetraacetate oxidation of the compound. These two glycosides have been found in a great number of red algae, most of the work being done by AUGIER and his group. Their results are briefly summarized in Table 1.

Table 1. Distribution of floridoside (F) and mannosidoglycerate (M) in red algae

Bangioideae

Order: *Bangiales* F, not isolated in a crystalline state, no M. Four orders not investigated.

Florideae

Order: *Nemalionales* F, no M.
,, *Gelidiales* F, no M.
,, *Cryptonemiales* F, the presence of M demonstrated or indicated in most algae.
,, *Gigartinales* F, the presence of M demonstrated or indicated in several algae.
,, *Rhodymeniales* F, the presence of M indicated in one alga of three investigated.
,, *Ceramiales* M, the presence of F demonstrated or indicated in several algae.

In the present investigation floridoside was isolated from *Porphyra umbilicalis*[18], *Gelidium cartilagineum*[19], *Furcellaria fastigiata*[18], *Gigartina stellata*[19], *Halosaccion ramentaceum*[19] and *Rhodymenia palmata*[19], and mannoside glycerate from *Ceramium rubrum*[19] and *Ptilota pectinata*[19], in agreement with the results given in Table 1. It is evident from the Table, however, that these

substances are not very useful from the taxonomical point of view. They are difficult to isolate in a state of purity when present in small amounts, and other methods to characterise them are not entirely reliable. It is also almost impossible to avoid a certain amount of impurities in the plant material and consequently the correctness of some of the deductions made as to the chemical content of the algae is perhaps open to doubt.

Two new glycerol glycosides were isolated during our investigations. The first, from *Furcellaria fastigiata*, *Gigartinales*, was proved to be an α-D-mannoside of floridoside[16, 18], the mannose being linked to oxygen at C_3 in the galactose part of the floridoside (VIII). The second, from *Porphyra umbilicalis*, *Bangiales*, was proved to be 1-glycerol α-D-galactopyranoside (IX)[18], and the name iso-floridoside is suggested for this compound. It occurs mixed with floridoside and this is probably the reason why the floridoside, isolated by AUGIER *et al.* from this and other algae of the same order, did not crystallise.

α,α-Trehalose has been isolated from some red algae and has now been obtained in low yield from *Bostrychia scorpoides*[19].

WATANABE and TAKANO[17] report the isolation of mannoside glycerate and a saccharide, which on hydrolysis yields galactose and rhamnose, from *Digenea simplex*.

4. *Internal salts*—Although beyond the scope of this survey, the isolation of some internal salts from red algae will be briefly mentioned[18]. Thus, taurine, N-methyltaurine and di-N-methyltaurine have been isolated. Unexpectedly, tri-N-methyltaurine was not found, but another quarternary ammonium salt, choline sulphate, was isolated. Red algae contain a high percentage of galactosane sulphate esters, and it seems to be a reasonable hypothesis that sulphate ester groups are transferred from choline to the polysaccharide by transesterification. A metabolic pathway from taurine (X) via mono-, di-, and tri-N-methyltaurine (XI) to choline sulphate might also be indicated by these results.

REFERENCES

1. AUGIER, J., and DU MERAC, M.-L., *Compt. rend.*, **238**, 238 (1954). AUGIER, J. ,*Compt. rend.*, **239**, 87 (1954) and preceding papers.
2. LINDBERG, B., *Acta Chem. Scand.*, **7**, 1119 (1953).
3. LINDBERG, B., and PAJU, J., *Acta Chem. Scand.*, **8**, 817 (1954).

4. LINDBERG, B., and MCPHERSON, J., *Acta Chem. Scand.*, **8**, 1547 (1954).
5. BOUVENG, H., and LINDBERG, B., *Acta Chem. Scand.*, **9**, 168 (1955).
6. LINDBERG, B., *Acta Chem. Scand.*, **7**, 1123 (1953).
7. LINDBERG, B., and MCPHERSON, J., *Acta Chem. Scand.*, **8**, 1875 (1954).
8. LINDBERG, B., *Acta Chem. Scand.*, **7**, 1218 (1953).
9. PEAT, S., WHELAN, W. J., and LAWLEY, H. G., *Chem. & Ind.*, 35 (1955).
10. HAAS, P., and HILL, T. G., *Biochem. J.*, **25**, 1470 (1931); **26**, 987 (1932).
11. HASSID, W. Z., *Plant Physiol.*, **8**, 480 (1933); **11**, 461 (1936).
12. COLIN, H., and GUEGUEN, E., *Compt. rend.*, **191**, 163 (1930).
13. PUTMAN, E. W., and HASSID, W. Z., *J. Amer. Chem. Soc.*, **76**, 2221 (1954).
14. COLIN, H., and AUGIER, J., *Compt. rend.*, **209**, 1450 (1939).
15. KAWAGUCHI, K., YAMADA, S., and MIYAMA, S., *Bull. Japan Soc. Sci. Fisheries*, **19**, 481 (1953); *Chemical Abstracts*, **49**, 4803 (1955).
16. LINDBERG, B., *Acta Chem. Scand.*, **8**, 869 (1954).
17. WATANABE, H., and TAKANO, T., *J. Pharm. Soc. Japan*, **73**, 529 (1953); *Chemical Abstracts*, **48**, 3259 (1954).
18. LINDBERG, B., *et al.*, *Acta Chem. Scand.* In press.
19. Unpublished results.

DISCUSSION

Dr. YOUNG asked Dr. LINDBERG if he considered mannitol glucoside or the galactosid as being related to the polysaccharides of the algæ. Dr. LINDBERG thought that this was so as mannitol glucoside had recently been isolated from partly hydrolysed laminarin. He found it very likely that the polysaccharides were synthetized from such glucosides. Dr. YOUNG asked Dr. LINDBERG if he thereby would suggest any relationship with inositol and glycerol, to which Dr. LINDBERG stated that too little evidence was gathered at that time.

Dr. BERGMANN, U.S.A., wanted to know how the algae were extracted. Dr. LINDBERG replied that the samples were extracted first with ether and then with methanol for a considerable time. The water soluble part of the methanol extract was ion exchanged and chromatographed on carbon or cellulose columns. Dr. BERGMANN said that during extraction of a *Fucus* species with acetone a crystalline compound separated which turned out to be the tri-acetone derivative of mannitol. He wanted to know if Dr. LINDBERG had made similar observations, but Dr. LINDBERG had not observed such phenomena. Dr. BERGMANN asked whether the taurine observed occurred as the free substance, which was verified by Dr. LINDBERG. To a question from Dr. BERGMANN about glycine Dr. LINDBERG answered that this substance would have been removed by ion exchange. Dr. BERGMANN pointed out that taurine had earlier been found only in certain starfish.

ON THE OCCURRENCE OF VITAMINS IN MARINE ALGAE

By H. Lundin and L.-E. Ericson (Sweden)

Read by H. Lundin

During the last few years, a series of investigations were carried out in this laboratory on the occurrence of vitamin B_{12} in different natural products. During these investigations, which were begun by Ericson and Banhidi[1, 2], it was shown that marine algae are often very rich in vitamin B_{12}. As was reported at the first International Seaweed Symposium in Edinburgh 1952[3], some algae were found to contain about 1 μg vitamin B_{12} per g dry weight.

It is known that a number of micro-organisms, e.g. several bacteria and streptomycetes can produce vitamin B_{12}. Whether vitamin B_{12} is formed by, or exists in higher plants is as yet uncertain, but if it is present it seems to occur in very small quantities. The high content of vitamin B_{12} in marine algae was therefore quite unexpected and may eventually be of practical importance. For the sake of comparison it can be mentioned that the animal organs, which are richest in vitamin B_{12}, often contain less than 1 μg per g dry weight.

After this high vitamin B_{12} content had been found, the investigations were continued on a larger number of algae[4–6]. It was shown that the occurrence of vitamin B_{12} is quite general. Of 25 marine algae studied, 23 contained detectable quantities of vitamin B_{12}. The quantitative variations were great, but as a rule brown algae seemed to be relatively poor in vitamin B_{12} (average 0·07 μg per g) while red algae were richer (average 0·27 μg per g) and, finally, green algae were the richest in the vitamin (average 0·35 μg per g). In the green alga, *Vaucheria dichotoma*, the content was as high as 2·8 μg per g. The difference in vitamin B_{12} content between brown, red and green algae is probably not of any taxonomic significance, but may be determined mainly by morphological characters (see below).

Many compounds closely related to vitamin B_{12} (cyanocobalamin) exist in Nature. The total 'vitamin B_{12} content' of the algae was obtained from microbiological analyses using *Escherichia coli* and *Lactobacillus lactis* Dorner. The growth of these organisms is stimulated not only by vitamin B_{12} but also by other components of the vitamin B_{12} complex. Chromatographic and electrophoretic separation of the B_{12}-like factors in algal extracts revealed that although most of their total 'vitamin B_{12} content' was due to vitamin B_{12} itself, these extracts also contained factor A[7, 8] pseudovitamin B_{12}[8, 9] and factor B[7, 8]. A new B_{12}-like factor called vitamin B_{12s} was also discovered[4].

An attempt was then made to find a likely explanation of the high vitamin B_{12} content of marine algae. The surfaces of many seaweeds are often covered with other organisms, e.g. algae, diatoms and bacteria. It was found that it is probably the bacteria living epiphytically on the seaweed that are the primary source of the vitamin B_{12}. Some of the facts that support this theory will be briefly mentioned here.

A number of bacteria were isolated from algae and from seawater in single cell culture[4]. Of 34 bacteria isolated 24 could produce vitamin B_{12}. The same B_{12}-like factors that were found in the algae were also produced by these bacteria. Bacteria from algae which were poor in vitamin B_{12}, generally produced small amounts of vitamin B_{12}, while bacteria from vitamin B_{12}-rich algae formed larger quantities of the vitamin.

Vitamin B_{12} seems to accumulate mainly in the cell walls of algae. Using the green unicellular alga *Valonia* it was easy to show this fact since the cell is very big. The cell wall was found to contain 0·2 μg vitamin B_{12} per g while the protoplasm contained less than 0·001 μg per ml. This indicates that the alga itself has not formed the vitamin but that this is derived from an external source.

In some other experiments algae were grown in glass tanks to which either radioactive cobalt (Co^{60}) or vitamin B_{12} containing Co^{60} had been added. These experiments were carried out in collaboration with the Institute for Seaweed Research, Inveresk, Scotland, by SCOTT and ERICSON[5–6]. The Co^{60}-labelled vitamin B_{12} was taken up and concentrated by the algae but only at the beginning of the experiment. Inorganic Co^{60}, on the other hand, was taken up during the whole of the experiment[5]. In one experiment

Co^{60}[6] was added to the water in a tank containing the algae *Rhodymenia palmata* and, after 17 days, an extract of the algae was made and examined using paper chromatography and paper electrophoresis. It was then found that the radioactivity could be separated from vitamin B_{12}. The algae had thus absorbed Co^{60}, but had not used it to synthesize vitamin B_{12}. The absorbed radioactive cobalt had been bound in another organic compound which was found to have strongly acidic groups, and from which the cobalt could be released with ascorbic acid.

All these investigations indicate that the vitamin B_{12} of marine algae is produced by the bacteria which grow on the algae or in the seawater and that the vitamin is then taken up and accumulated by the plant. The amount of vitamin B_{12} in an alga would therefore be determined mainly by two factors: (1) the type of bacteria growing on the surface or in the immediate surroundings of the alga; (2) the ability of the alga to concentrate the vitamin. As already stated green algae are generally richer in vitamin B_{12} than red algae, and the latter are richer than brown algae. One reason for this could possibly be that green and red algae often have greater surface areas per gram dry weight than the brown algae.

There are large seasonal variations in the algae's vitamin B_{12} content[4]. In *Alaria esculenta**, for instance, the vitamin B_{12} content increases five fold from February to May. It then decreases from May to August and is then barely half the February value.

Marine algae have also been shown to contain several different water-soluble growth factors related to the folic acid and the folinic acid groups of vitamins[10]. Algae representative of the four classes *Rhodophyceae*, *Phaeophyceae*, *Chlorophyceae* and *Heterokontae* have been investigated. Folic acid acitivity was measured microbiologically using *Streptococcus faecalis* ATCC 8043 in an agar cup plate method. Folinic acid was determined with *Leuconstoc citrovorum* ATCC 8081 in the medium of Bolinder *et al*[11]. again using the agar cup plate method. Pteroylglutamic acid was used as standard for *S. faecalis* and N_5-formyl, 5,6,7,8-tetrahydrofolic acid (folinic acid) for *L. citrovorum*.

* Collected at Lofoten from August 1950 to August 1951. The algal samples were kindly supplied by Mr. A. Haug, Norsk Institutt for tang og tareforskning Trondheim, Norway.

The folic acid activity is generally of the order of 0·5–1·0 μg per g dry weight. It is very low in *Ascophyllum nodosum*, 0·07 μg per g and high in *Chara tomentosa*, 15 μg per g dry weight. The folinic acid activity of the algae is with few exceptions lower than the folic activity varying from 0·07 to 1·1 μg per g. The differences between the folic or folinic acid activity of fertile tips and the rest of the algae are generally small. As was expected great seasonal variations were found in the total folic and folinic acid activity. In *Alaria esculenta* the folic acid activity appears to be high during the spring and the summer but low during the autumn. The folinic acid activity curve has a sharp maximum in January. In contrast to the observed variation in folic acid activity, no increase in folinic acid activity could be noticed in the spring.

A number of algae were also analysed for niacin and pantothenic acid[12]. For the microbiological determination of these vitamins *Lactobacillus arabinosus* 17-5 was employed. The amounts of niacin ranged between 1 μg per g for *Ceramium tenuicorne* to 63 μg per g dry weight for *Alaria esculenta*. The pantothenic acid contents varied greatly: from less than 0·2 μg per g in some algae up to 12·5 μg per g dry weight in *Chara tomentosa*. The seasonal variation in these two vitamins seems to be somewhat less pronounced than that observed for vitamin B_{12}, folic and folinic acid.

SUMMARY

The vitamin content of marine algae undergoes marked seasonal variation and no simple rule can be given for the time of harvesting of algal samples with a 'high vitamin content'. The concentrations of some vitamins show maxima in the spring and the summer (e.g. vitamin B_{12} and folic acid) whereas other vitamins have their concentration maxima in the winter (e.g. niacin and folinic acid). The content of vitamin B_{12}, folic acid, folinic acid and pantothenic acid (and also of vitamin B_1[13]) is generally higher in the green and the red algae than in the brown algae. The niacin (and vitamin C[14]) content appears to be more or less the same in red, brown and green algae.

From these studies and from the investigations of other authors [13,14] it can be concluded that many marine algae are good sources of vitamins.

REFERENCES

1. L.-E. ERICSON and Z. G. BÀNHIDI, *Acta Chem. Scand.*, **7**, 167 (1953).
2. Z. G. BÀNHIDI and L.-E. ERICSON, *Acta Chem. Scand.*, **7**, 713 (1953).
3. L.-E. ERICSON, Z. G. BÀNHIDI, A. G. M. SJÖSTRÖM, and H. LUNDIN, *Proc. First International Seaweed Symposium*, Edinburgh (1952).

4. L.-E. Ericson and L. Lewis, *Arkiv Kemi*, **6**, 427 (1953).
5. L.-E. Ericson, *Chem. & Ind.*, 829 (1952).
6. R. Scott and L.-E. Ericson, *J. Exptl. Botany*, **6**, 348 (1955).
7. J. E. Ford, S. K. Kon and J. W. G. Porter, *Chem. & Ind.*, **22**, 495 (1952).
8. E. S. Holdsworth, *Nature*, **171**, 148 (1953).
9. J. J. Pfiffner, M. W. Dion, and D. G. Calkins, *Federation Proc.*, **11**, 269 (1952).
10. L.-E. Ericson, *Arkiv Kemi*, **6**, 503 (1953).
11. A. Bolinder, E. Widoff, and L.-E. Ericson, *Arkiv Kemi*, **6**, 487 (1953).
12. L.-E. Ericson and B. Carlson, *Arkiv Kemi*, **6**, 511 (1953).
13. G. Gerdes, *Arch. f. Mikrobiologie*, **16**, 53 (1951).
14. S. Rönnerstrand, *Untersuchungen über Oxydase, Peroxydase, und Ascorbinsäure in einigen Meeresalgen*, C. W. Lindström, Lund 1943.

DISCUSSION

Dr. Bergmann, U.S.A., asked if the B_{12} was believed to be produced by the bacteria and microorganisms on the surface of the algae and then absorbed by the algae. Dr. Lundin stated that the vitamin partly remained in the bacteria and partly was absorbed on the surface of the algae. Dr. Bergmann pointed out that the vitamin B_{12} content of the microorganisms growing on the surface might otherwise be a rather staggering figure and wanted to know if any of these organisms had been isolated. Dr. Lundin answered that 34 had been isolated and 24 of these were capable of B_{12}-production.

Dr. Young, Canada, inquired about the possibility of any symbiosis between particular bacteria and the particular alga involved, but Dr. Lundin could give no information on this point.

VARIATIONS IN THE CHEMICAL COMPOSITION OF SPANISH BROWN SEAWEEDS

By C. Primo (Spain)

Read by J. M. Perello (Spain)

ABSTRACT

A study has been made of the total ash content, alginic acid, zinc and copper contents of seaweeds found on the Spanish coasts. Special attention has been paid to seasonal variations in *Laminaria digitata* and *Fucus spiralis* at Santander (Biscay coast), during the years 1946–47 and 1951–52.

The total ash content, determined by calcination, for *L. digitata* was: a maximum in February and March of 33–38% and a minimum in July and August of 27%. For *F. spiralis*, two maxima were obtained for March and September of 32% and 24% respectively; and minima in July and November of 20 and 22%.

The alginic acid content was determined by the Cameron-Ross-Percival technique. For *L. digitata*, a maximum in April and March of 27% and a minimum in October of 16%. In the case of *F. spiralis*, two maxima were obtained for March and September of 17 and 14·5% and minima in June and November of 12·5 and 10%.

Zinc and copper contents were obtained by polarography. In the case of *L. digitata*, for zinc, a maximum of 210 ppm in December and a minimum of 105–108 ppm for March and June. Copper: a maximum of 21 ppm in January and a minimum of 9 ppm for April. For *F. spiralis* the maximum zinc content was 230 ppm for August and there was a minimum of 155 and 160 ppm for April and October. For copper, the maximum was 16 ppm for August and the minimum 3–5 ppm for March and April.

DISCUSSION

To a question by Dr. Andersen about the form of the copper in the algae, Dr. Perello answered that Dr. Primo had not investigated this point.

STUDIES ON THE HETEROGENEITY OF CARRAGEENIN

By David B. Smith, A. N. O'Neill and A. S. Perlin (Canada)

Read by E. Gordon Young (Canada)

ABSTRACT

Carrageenin has been separated into a potassium sensitive fraction (designated κ-carrageenin) and a potassium insensitive fraction (designated λ-carrageenin).

Except for a trace of xylose, the only carbohydrate residues in κ-carrageenin are 3,6-anhydro-D-galactose and D-galactose which are present in nearly equal proportions. Tests following fractional precipitation of κ-carrageenin indicate general chemical homogeneity but physical heterogeneity. κ-carrageenin is not susceptible to periodate oxidation. Its sulphate content is about 25%.

Fractional precipitation of λ-carrageenin with ethanol separates a main fraction in which only D-galactose and a trace of 3,6-anhydro-D-galactose have been found together with about 30% esterified sulphate. This polysaccharide is susceptible to periodate oxidation and hence cannot be a linear polymer of D-galactose-4-sulphate. It is polydisperse on a mass basis.

Material containing glucose, xylose and L-galactose separate into minor fractions of λ-carrageenin and may belong to contaminating floridean starch, xylan and an L-galactose containing polysaccharide.

L-galactose has been isolated from carrageenin by a simple procedure.

(*For Discussion, see page* 3)

Section II

STUDIES WITH SPECIAL REFERENCE TO PRACTICAL USES

THE PRODUCTION OF ANTIBIOTIC SUBSTANCES BY SEAWEEDS

By C. G. C. Chesters and J. A. Stott (England)

Read by C. G. C. Chesters

Pratt, Mautner *et al.*, reported the antibiotic acitivity of extracts of certain American seaweeds in 1951. In 1954, Vacca and Walsh demonstrated that an ethyl alcohol extract of *Ascophyllum nodosum* possessed inhibitory activity, and in 1953 Mautner, Gardner and Pratt, dealing with an active extract from *Rhodomela larix*, suggested that the activity might be due to a brominated phenol.

Since early spring 1953 we have been examining the antibacterial activity of extracts of British seaweeds. Preliminary experiments made it clear that the most favourable extractant was ether. The quality of this substance varies for different commercial brands and careful control tests of the ether itself are essential before evaluating assay tests. The final standard routine for extraction consists of collecting fresh weed, freezing in solid CO_2 in thermos jars within 30 minutes of collection, storing in a deep freeze at $-18°$ C to $-20°$ C, thawing and rinsing in fresh water and preparing for extraction. Preparation for extracting is carried out either by freeze-drying the washed sample, or by oven-drying initially at 80° C (weed temperature) for 2–3 minutes and finishing at 60° C to 70° C (weed temperature) for 4–5 hours. Control experiments have shown that no activity is lost in oven-dried material as compared with freeze-dried material. Soxhlet extraction in ether for 24 hours is followed by concentration of the extract to such a volume that 0·1 ml extract represents the soluble material from 0·2 g of dried weed. Thus 0·1 ml. extract represents the soluble material from about 1·6 to 2·0 g wet weed.

Assay is carried out by placing sterile filter paper discs containing 0·1 ml. concentrated extract on seeded plates or by placing strip chromatograms on such plates. Ether controls with the same volume content and run as blanks through the routine extraction are incorporated in every assay test. For both methods values are

read after incubation at 26° C (37° C for *B. coli*) for 18 hours and are expressed as diameters in millimetres for discs and as square millimetres for chromatograms.

The range of seaweeds examined is shown in Table 1. Negative reactions are quite definite; doubtful reactions mainly refer to early

Table 1

		1	2	3	4	5	6	7	8	9	10	11	12
ASCOPHYLLUM	3				?								
	4					⊖							
	5												
FUCUS SERRATUS	3				?								
	4												
	5												
" SPIRALIS	3					⊖							
	4												
	5												
" VESICULOSUS	3				⊖			⊖					
	4												
	5												
HALIDRYS	3			?						⊕			
	4				⊕	⊕			⊕				⊕
	5				⊕								
PELVETIA	3						?						
	4					⊖			⊖				
	5												
LAMINARIA DIGITATA	3			?						⊕			
	4				⊕	⊕			⊕				
	5												
" SACCHARINA	3			⊖								⊖	
	4												
	5												
HIMANTHALIA	3					?							
	4												
	5												
CHORDARIA	3										⊖		
	4												
	5												
PORPHYRA	3				?								
	4												
	5						⊖						
CERAMIUM	3					?							
	4												
	5				⊖								
LAURENCIA	3									⊖			
	4												
	5												
POLYSIPHONIA	3				?								
	4	⊕			⊖	⊕			⊕				⊕
	5					⊕							
FURCELLARIA	3					?							
	4												
	5				⊕								
GIGARTINA	3			?	⊖								
	4				⊖	⊖							
	5												
RHODYMENIA	3										?		
	4												
	5												

work (1953) and experience suggests that some at least should be positive if present day routine were applied during extraction; positive results are such that there is no doubt about the activity of the extract. Table 2 shows the distribution of activity in seaweed species against the test organisms. *Halidrys siliquosa*, *Pelvetia canaliculata*, *Laminaria digitata* (fronds) and *Polysiphonia fastigiata*

give clearly positive results. In Table 3 actual values are set out (diameters in millimetres for discs in the left-hand column, areas in square millimetres for chromatograms in the right-hand column). *Polysiphonia fastigiata* shows the widest bacterial spectrum and activity against *Pseudomonas pyocyanea* is interesting. For

*Table 2**

	ESCH. COLI	PS. PYOCYANEA	BACT AEROGENES	B. SUBTILIS 13	" " 30	STAPH. ALBUS	PS. FLUORESCENS	CORYNEFORM 13	" 18	ACHROMOBACTER 6	" 17	B. PUMILIS
ASCOPHYLLUM	▦	▦	▦	═		▦	▦	═	═	═	═	═
FUCUS SERRATUS	═	═	═	▦		═	▦					
" SPIRALIS	═	═	═		═	═	═					
" VESICULOSUS	═	═	═	═	═	═	═					
HALIDRYS	═	═	═	═	⊞	═	═	⊞	═	═	⊞	⊞
HIMANTHALIA	═	═	═	═	▦	▦	═					
PELVETIA	═	═	═		⊞	═	▦	⊞	═	═	═	═
LAMINARIA DIGITATA	═	▦	▦	▦	⊞	▦	▦	⊞	⊞	═		⊞
" SACCHARINA	═	═	═	═	═	═	═	═	═	═	═	═
CHORDARIA	═	═	═		═	═	═					
PORPHYRA	▦	▦	▦	▦		▦	▦					
CERAMIUM RUBRUM	═	▦	═		▦	═	▦		═			
LAURENCIA	▦	▦	▦		▦	═	▦					
POLYSIPHONIA	▦	⊞	▦		⊞	⊞	⊞	⊞	⊞	⊞	⊞	⊞
FURCELLARIA	═	▦	═		▦	═	▦	═	═	═	═	⊞
GIGARTINA	═	▦	═	▦	═	═	═	═	═	═	═	═
RHODYMENIA	═	▦	▦		▦	▦	▦					

each of these weed species activity decreases in certain months, and may even be absent. There is evidence that this is a general feature of all seaweeds. Insufficient data is available to justify detailed

* Large squares = positive, small squares = doubtful, bars = negative, and blanks = no test.

FOOD TECHNOLOGICAL RESEARCH INSTITUTE
LIBRARY

Table 3

		1953				1954				1955	
		April	May	January	April	May	August	December	April	May
Halidrys	*B. subtilis*		750 500		19·9 19·4	19·0 18·5	trace	19·6 19·6	18·6 18·3	
	Coryneform 13		21·0 330 20·3 330		19·5 20·0	19·0 19·9	trace	trace	22·5 21·8	
	B. pumilis				19·1 19·3	0	21·6 21·3	0		
Laminaria digitata	*B. subtilis*		918 368		21·0 20·8	21·0 20·8	trace	18·8 19·0		
	Coryneform 13		961 1346		19·8 19·5	19·8 19·8	trace	trace		
	B. pumilis,				19·2 19·2	19·2 19·2		19·1 19·3		
Polysiphonia fastigiata	*Ps. pyocyanea*	21·0 21·3		18·5 18·5	0	0	22·6 22·7	0		19·6 19·6
	B. aerogenes	12·5 12·3	0	0	0	0	0	0		0
	B. subtilis	22·0 1140 22·5 1140		19·0 433 18·5 462	0		20·8 21·0	0		18·8 18·9
	Staph. albus	23·3 750 21·3 800		614 0 593	0		22·7 22·0	19·8 20·2		19·6 19·5
	Ps. fluorescens	23·7 800 23·7 1135		19·0 289 19·0 271	0		21·2 21·0	0		18·5 18·5
	Coryneform 18			160 168	0		19·3 19·6	0		
	Achromobacter 6						30·3 30·0	21·2 22·7		
	,, 17						29·1 30·7	24·8 25·5		
	B. pumilis						23·7 26·1	25·1 24·1		

Fig. 1.

FIG. 2.

FIG. 3

FIG. 4.

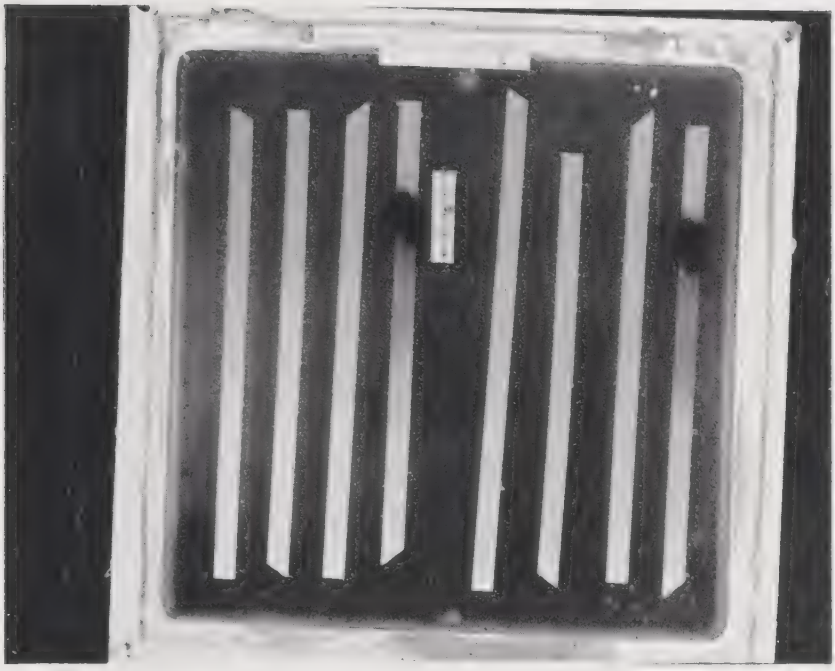


Fig. 5.

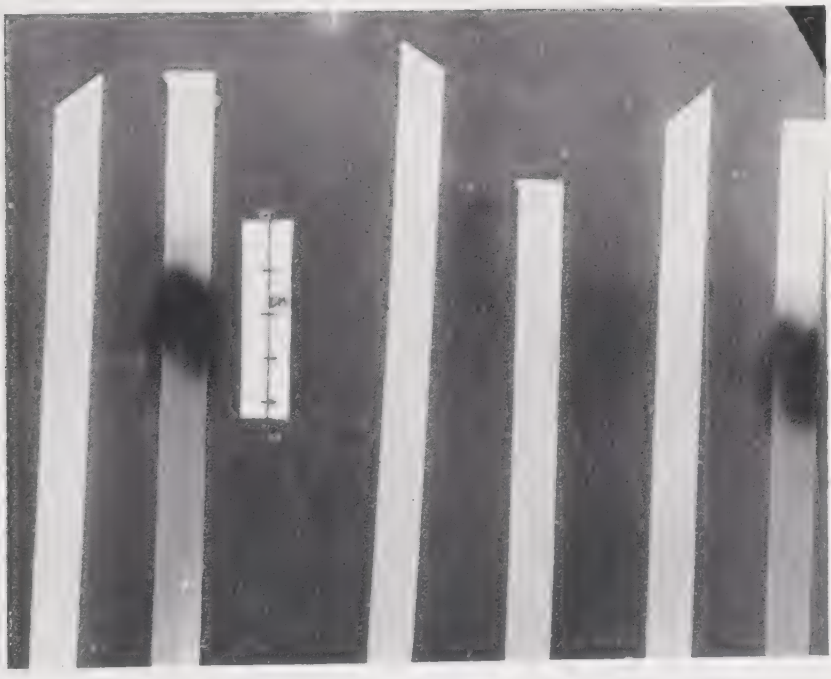


Fig. 6.

comment on this phenomenon but it would appear that each seaweed has a different periodicity.

An indication of the degree of activity to be expected from disc assays of an extract of *Polysiphonia* is given in Fig. 1 for a gram negative *Chromobacterium sp.*, member of the *Achromobacteriaceae* and in Fig. 2 for *Arthrobacter sp.*, a Coryneform species. Figs. 3 and 4 show the reaction of a chromatogram and its control against *Pseudomonas pyocyanea*, the extract being from *Rhodymenia palmata* run with ether and reversed with acetone. Fig. 5 shows a similar series of strip chromatograms using a *Polysiphonia* extract against *Pseudomonas fluorescens*. The active area is enlarged in Fig. 6.

Chromatographic treatment of ether extracts demonstrates that the active material is associated with the green pigments. Whether it is intimately and closely associated with the chlorophylls is still uncertain but the fact that it only occurs in certain seaweed species suggests that it should be separable. From the fact that the extracts of *Halidrys* are not active against *Pseudomonas pyocyanea*, there is at least an indication that the active substance here may be different from that produced by *Polysiphonia* and certain other *Rhodophyceae*.

REFERENCES

Pratt, R., Mautner, H., Gardner G. M. G., Sha, yi-Hsien and Dufrenay, J., Antibiotic Activity of Seaweed Extracts, *J. Amer. Pharm. Ass.* (*Sci. Ed.*), **40**, 575 (1951).

Mautner, H., Gardner, G. M. G. and Pratt, R. Antibiotic Activity of Seaweed Extracts, II, *Rhodomela larix.*, *J. Amer. Pharm. Ass.* (*Sci. Ed.*), **42**, 294–6 (1953)

Vacca, D. D., and Walsh, R. A., The Antibacterial Acitivity of an Extract Obtained from *Ascophyllum nodosum*, *J. Amer. Pharm. Ass.* (*Sci. Ed.*), **43**, 24–26 (1954).

DISCUSSION

Dr. Wood, U.S.A., wanted to know if any of the antibiotics had been tested against viruses at all, or if they had been used in animals or human beings. But the speaker had not tested the antibiotics in this way.

Dr. Isaac, South Africa, asked if Dr. Chesters had obtained an antibiotic extract of male, female and tetrasporic plants of *Polysiphonia* or only from one or a mixture of these phases, or if there was any difference in yield depending on environmental conditions. Dr. Chesters stated that they had not been able to observe any environmental influences and the possible variation from generation to generation had not been fully investigated.

Dr. HOFFMAN, Germany, asked whether Dr. CHESTERS had found any water soluble antibiotic substances, as investigations in Kiel had revealed the presence of water soluble active substances from *Fucus*, *Rhodomela*, and *Delesseria*. Dr. CHESTERS stated that they had been investigating water soluble antibiotics from certain algae but owing to the great variations and to difficulties with the control he was not willing to say that he had ever seen any activity of water extracts.

Dr. ROMIJN, Holland, wanted to know if the substances had been tested against bacteria of the *Salmonella* group and whether growth experiments with these compounds on young animals had been carried out. Dr. CHESTERS repeated that no animal experiments had been undertaken. No adequate investigations with *Salmonellas* had been carried out.

Mr. JENSEN, Norway, pointed out the difficulties caused by the microorganisms adhering to the algae and wanted to know if there was any evidence for the antibiotic compound being produced by the algae and not by the microorganisms. Dr. CHESTERS answered that the point had not been fully investigated but that the amounts of extracts seemed to indicate that the antibiotics could not possibly in any way be related to the surface-growing organisms.

Mrs. KYLIN, Sweden, mentioned that she had observed an antibiotic effect of certain green algae (*Platymonas*) and asked Dr. CHESTERS if he had tested unicellular green algae, but Dr. CHESTERS had not investigated any of these algae yet.

Mr. FLOOD, Ireland, raised the question of the activity observed in the Soxhlet thimbles and wanted to know to what this could be attributed. Dr. CHESTERS replied that by treatment of the Soxhlet thimbles with boiling water a clay-like precipitate was formed, which was quite toxic to certain bacteria.

SODIUM LAMINARIN SULPHATE AS A BLOOD ANTICOAGULANT

By E. T. Dewar (Scotland)

INTRODUCTION

The natural anticoagulant, heparin, is the sulphuric acid ester of a polysaccharide composed of glucuronic acid and glucosamine residues, although the complete structure is not yet clear. When it was realised that the activity of heparin depended on the presence of sulphuric acid ester groups in the molecule, various attempts were made to find a substitute by introducing sulphate residues into other polysaccharides. Consequently, the literature during the past 20 years contains many examples of polysaccharide sulphuric esters exhibiting heparin-like activity *in vitro*, e.g. cellulose, starch, glycogen, xylan, inulin, chitin, alginic acid, etc., but most of these active preparations have been proved unsuitable for clinical use because in comparatively low dosage they produced, in laboratory animals, a tendency to bleeding. They form insoluble complexes with the plasma-proteins, especially fibrinogen, and cause other undesirable pathological changes.

In 1945, in Sweden, sulphuric esters were prepared from dextran, which is a glucose consisting largely of α-D-glucopyranose units linked through the 1 : 6-positions, and it was found, by using dextrans of various molecular weights, that the toxicity declined as the molecular weight decreased. This work on dextran sulphate has been continued in England during the past 6–7 years by Ricketts and Walton, and they have shown that activity is more or less independent of molecular weight but depends on a certain minimum sulphur content; at 1·0–1·3 sulphate groups per glucose unit the activity increased to its maximum value of about 15 heparin units per mg. But they also found, in agreement with the Swedish workers, that toxicity is directly related to molecular weight, and osmotic pressure measurements indicated that the optimum molecular weight of non-toxic dextran sulphate was of the order of 7,000. In its native state, dextran is a very large mole-

cule containing many thousand glucose units in its branched structure, and in order to obtain material suitable for a non-toxic sulphate ester the molecule must be degraded by careful acid hydrolysis and the high molecular fractions separated by fractional precipitation with acetone. The sulphate itself must also be fractionated in order to pass the strict clinical specification laid down by the British Medical Research Council.

It occurred to us that laminarin, the 1 : 3-β-polyglucose of brown algae, might yield, without any degradation or fractionation, an active, non-toxic sulphate ester, since its molecular weight has recently been shown to be in the 3500–5000 range. Laminarin, a free-flowing white powder which has been successfully used as a dusting powder for surgeons' gloves, occurs at certain times of the year to the extent of 35% of the dry matter of *Laminaria cloustoni* frond growing in Scottish waters. It is easily extracted from fresh or dried milled seaweed by means of dilute acid and separates as a pure insoluble precipitate from this aqueous acid solution. The sulphate ester is prepared in the usual way with chlorosulphonic acid in pyridine, and by varying the proportion of chlorosulphonic acid in the reaction mixture a series of laminarin sulphates, containing from 0·6 to 2·2 sulphate groups per glucose unit, has been prepared (Table 1). When the sulphate content falls below about 30%, aqueous solutions of these esters become unstable and start to precipitate on standing (cf. samples D and E). It has been found

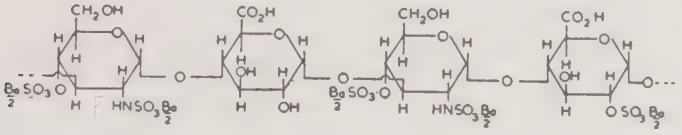
Table 1. Analysis of various sodium laminarin sulphates

Laminarin sulphate	Chlorosulphonic acid per 5 g of laminarin, ml	Reaction time at 65–70°, min.	Ash (as Na_2SO_4), %	Sulphate in ash (as SO_4), %*	Total sulphate on hydrolysis (as SO_4), %	No. of sulphate groups per glucose unit	Yield, %	Stability of 6% aqueous solution	$[\alpha]^{18}_D$ in water (c, 2·0)
A	8·8	75	41·6	28·1	54·1	2·15	99·4	no pptn. after 32 days	−9·8°
B	6·0	30	41·7	28·2	52·9	2·04	104·6	—	—
C	4·0	30	32·3	21·8	43·5	1·37	86·2	no pptn. after 32 days	−3·7°
D	2·0	30	19·6	13·2	26·1	0·61	87·1	18·2% pptd. after 11 days	−8·2°
E	1·0	15	7·4	5·0	10·5	0·20	78·2	47·7% pptd. after 6 days	—

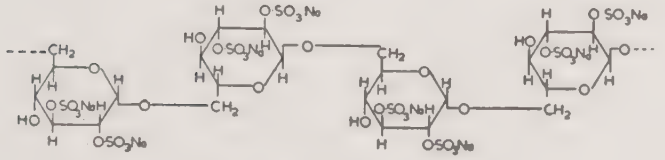
* Calculated from the ash.

that the product containing two sulphate groups per glucose unit gave maximum activity of about 40 units per mg.

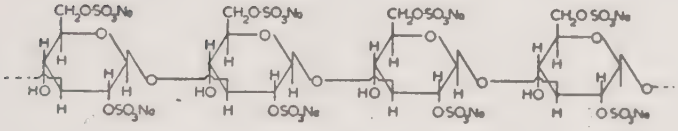
The reason for the high anticoagulant activity of heparin compared with that of other sulphated polysaccharides is still not clearly understood, although the structure of heparin differs considerably from most other polysaccharides. The repeating unit probably consists of alternating glucuronic acid and glucosamine residues with the amino groups of the latter sulphated (Fig. 1).



HEPARIN ($B_{\frac{1}{2}}$ SALT).



DEXTRAN SULPHATE (N_a SALT)

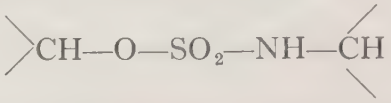


LAMINARIN SULPHATE (N_a SALT).

FIG. 1.

While the presence of sulphate groups is essential for activity it is not the only factor involved, for the sulphur content of heparin is less than that of laminarin sulphate or dextran sulphate containing two sulphate groups per glucose unit. It has been suggested that the N-sulphate groups on the glucosamine residues are largely responsible for activity, for laminarin and dextran sulphates contain only O-sulphate groups; but it has been shown recently that loss of activity on mild acid treatment is not due to the release of free amino groups. Furthermore, RICKETTS has found that sulphation of

deacetylated chitin does not lead to a highly active material although it contains sulphated amino groups attached to glucosamine residues as in heparin. It is likely that the activity of heparin is due to a combination of several factors, e.g. a small proportion of the sulphur may be present as a sulphate bridge:



which may profoundly affect molecular size and shape.

ESTIMATION OF LAMINARIN SULPHATE IN URINE AND BLOOD

In order to follow the urinary excretion of laminarin sulphate after injection into experimental animals, a colorimetric method based on the metachromatic reaction with toluidine blue was worked out. A calibration curve was drawn up by treating 10 ml. standard solutions, containing 0–30 μg. laminarin sulphate, with exactly 1 ml. of 0·01% toluidine blue solution and measuring the colour intensity at 630 mμ in a Unicam SP. 600 Spectrophotometer; the graph of optical density against concentration is shown in Fig. 2. To determine laminarin sulphate in urine, the specimen

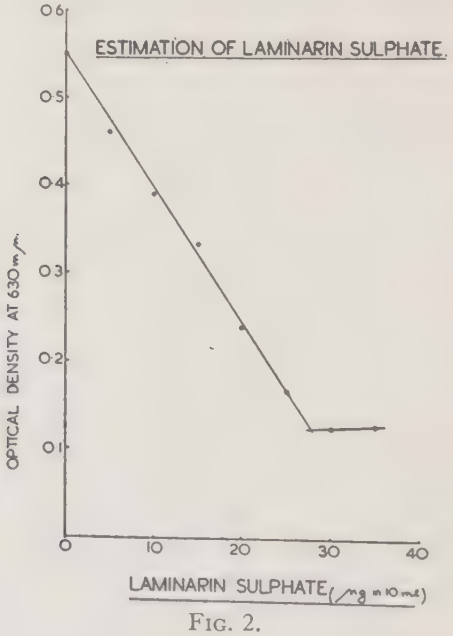


Fig. 2.

was diluted until 10 ml. of diluted solution gave a colour which lay on the graph. Within the optical density range 0·25–0·45 the accuracy is of the order of ± 10%.

The estimation of laminarin sulphate in plasma is not so accurate. It is well known that heparin in blood cannot be detected with toluidine blue, the sulphate radicals being modified in some way by the plasma proteins so that the red-purple, metachromatic colour is not developed. It was found, however, that laminarin sulphate in rabbit plasma did give the reaction, although the characteristic metachromatic colour was not so intense as in water or urine and optical densities were never less than 0·20 (Fig. 2). A semi-quantitative estimation could be obtained by suitable dilution of the plasma to give an optical density within the range 0·35–0·45; the accuracy was of the order of ± 30%. Much longer times are required for maximum colour development in the case of plasma.

FRACTIONATION OF LAMINARIN SULPHATE

In an attempt to relate molecular size to biological properties,

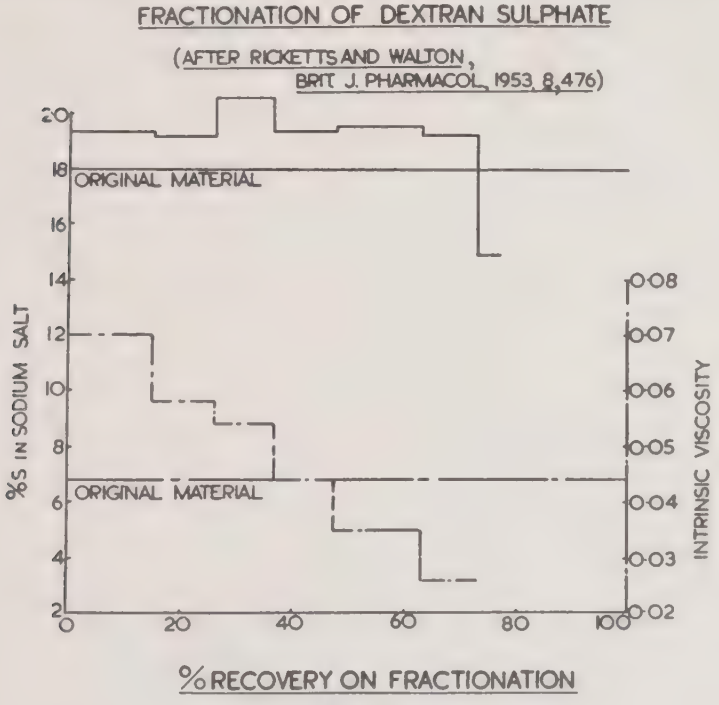


Fig. 3.

RICKETTS and WALTON subjected dextran sulphate to fractional precipitation from ethanol-water solution. Fig. 3 shows how the original material, containing 17·9% sulphur and an intrinsic viscosity of 0·044, was separated into seven fractions (77% recovery), the sulphur contents of which varied between 20·6 and 14·9% and the intrinsic viscosity between 0·070 and 0·026. Activity decreased with decreasing intrinsic viscosity in the range 0·054–0·026, and only material with an intrinsic viscosity of 0·035 and less was completely free from undesirable properties; fractions with a higher viscosity gave precipitation of fibrinogen and a positive anaphylactoid reaction in guinea-pigs. This latter test is of importance, for the Medical Research Council has stipulated that clinical dextran sulphate must pass this test.

Fig. 4 gives the results obtained on fractionation of laminarin sulphate under the same conditions. 97·7% of the initial material

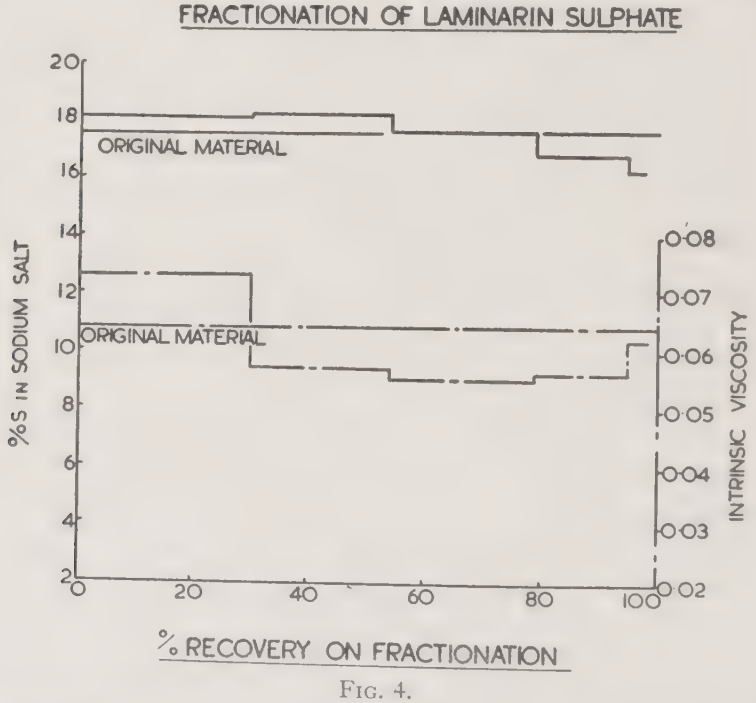


FIG. 4.

was recovered at 51% ethanol concentration and, although variations do occur in sulphur content and intrinsic viscosity, they are appreciably less than for dextran sulphate. With the exception of fraction 1, which would appear to contain slightly higher molecular

material, the intrinsic viscosities indicate that the remaining material is essentially homogeneous.

Laminarin sulphate, containing two sulphate groups per glucose unit, is a white powder, which is stable for long periods if maintained in the anhydrous condition. For injection, a 17% solution in 0·65% sodium chloride is buffered with a small quantity of sodium bicarbonate and sterilised by autoclaving at 10 lb./in.^{2} for ten minutes. The clear amber solution has passed rigorous tests for stability. Extensive trials with rats, rabbits and dogs have been carried out at the Department of Surgery in the University of Edinburgh, but complete results are not yet available.

DISCUSSION

Dr. Percival, Scotland, asked Dr. Dewar if the experiments were carried out on the insoluble fraction, and Dr. Dewar answered that this was correct. Dr. Percival then wanted to know if the speaker had any evidence for the molecular weight suggested. Dr. Dewar said he was only quoting the figures given for insoluble laminarin. Dr. Percival's questions were based on some observations made in Edinburgh on methylated laminarin indicating that a fraction of the polysaccharide might have a molecular weight over 10,000.

Dr. Young, Canada, stated that they had been working on the sulphate of soluble laminarin and he would like to know if anyone had observed any toxic effects with these compounds, and would also like to know what sort of tests Dr. Dewar had used. The speaker answered that in the very sensitive animal tests used by them, certain effects of a toxic type were observed, e.g. a positive anaphylactoid reaction in guinea pigs.

Dr. Woodward raised the question of the molecular weight of laminarin and indicated that the differences in behaviour of the polysaccharide might be a result of differences in the techniques used.

THE VITAMIN CONTENT OF ALGAE—AND ITS SCIENTIFIC CONSEQUENCES

By Poul Freudenthal (Denmark)

ABSTRACT

The first fundamental and exact biological investigations on vitamin A in three Danish marine algae are mentioned.

The experimental diet was pure, synthetic, adequate and sufficient so that a single deficiency factor at a time could be dealt with.

The standards of investigation were: histological examination of the bones and analysis of the water content, dry matter, and ash from the bones of a single experimental animal.

Doses of finely ground algae were given on a scale of from 0·025 to 1 gr. daily. Experimental animals, rats, that had had no algae or a dose of 0·025 showed arrest of growth, osteoporosis, and xerophthalmia. A dose of 0·05, 0·10 (and 0·20) caused rickets, some growth, but no xerophthalmia. A dose of 0·30 and over caused normal development.

An admixture of 'a therapeutic dose of vitamin D' showed no favourable effect and had no antirachitic effect. Animals receiving a dose of 0·30 of finely ground algae were fully fertile and capable of breeding though there was no fat or other supposed vitamin in the diet.

A dose of 200 gr. to milk-producing cows caused an increased yield of milk, increased fat percentage, and increased vitamin A content in the butter-fat.

A dose of 25 gr. in pig-breeding showed full equivalence to lucerne green flour and carrot flour.

The consequences for the recognition by medical science of the etiology, pathogenesis and therapy of rickets are mentioned. The current conception of the concepts 'D' and 'E' vitamins, and the theories of ultra-violet light are criticised, and finally the importance hereof for the nation's food is mentioned.

(*For Discussion, see page* 22)

A STUDY OF SEAWEED DRYING

By R. G. Gardner and T J. Mitchell (Scotland)

Read by R. G. Gardner

This paper outlines the development of a drying process for freshly harvested *Laminaria cloustoni*.

Preliminary tests in which the stipe and frond were tested separately in pneumatic, rotary-louvre and through-circulation dryers indicated that chopped stipe could be readily dried in any of the three types but, owing to its sticky nature, frond could only be dried satisfactorily in the through-circulation dryer. Further investigation was, therefore, confined to this type of dryer.

Extensive laboratory tests on a through-circulation dryer using sliced stipe or minced frond showed that there is a critical bed depth for frond at which the output is at a maximum. Other factors investigated included temperature, humidity and velocity of the air stream, particle size and agitation of the bed.

The drying rate of a bed of seaweed was found to be approximately proportional to the wet-bulb depression of the inlet air and this observation was used to devise a graphical design method for estimating the drying time of seaweed in a continuous multistage conveyor dryer.

Recent work has shown that littoral seaweeds have similar drying characteristics to *L. cloustoni* frond.

Nomenclature

T	= total water content, lb. water/lb. B.D.S. = the water ratio.
θ	= drying time, min. (usually for $T=5$ to $T=0{\cdot}15$).
L	= dry loading, lb. B.D.S./sq.ft.
G	= mass air flow, lb. dry air/(min.)(sq. ft. of bed area).
S	= slice thickness, in.
R	= output rate, lb. C.D.S./(sq.ft.)(hr.).
D.B.T.	= dry-bulb temperature, °F.
W.B.T.	= wet-bulb temperature, °F.
W.B.D.	= wet-bulb depression, °F = D.B.T. − W.B.T.
B.D.S.	= bone-dry solid.
C.D.S.	= commercial dry solid.

1. INTRODUCTION

Thermal drying of seaweed has the advantages of reducing transport costs of the product, of enabling the material to be stored

without bacterial attack and of allowing it to be readily utilized in other products, e.g. animal feeding stuffs.

Non-thermal processes such as ensilaging or centrifuging while cheaper than drying do not render the seaweed suitable for storage and transport.

This paper outlines the development of a drying process for *Laminaria cloustoni* harvested from the coast of Scotland (Oban) about 24 hours before drying. The initial water content of the seaweed (dry basis) ranged from about 4–6 lb. water/lb. B.D.S. for stipe and from 2·5–7 lb.lb B.D.S. for frond.

2. PRELIMINARY SELECTION OF DRYER TYPES

A critical survey of various types of dryer was made on the basis of output, capital cost, thermal efficiency and labour requirements. The most critical factor appeared to be the sticky nature of the frond particles.

It was concluded that the rotary-louvre, pneumatic and through-circulation dryers were the most promising and seaweed drying tests were carried out on representative dryers of these types.

2.1. *Rotary-Louvre Dryer*

The rotary-louvre dryer (Fig. 1) at the Institute of Seaweed

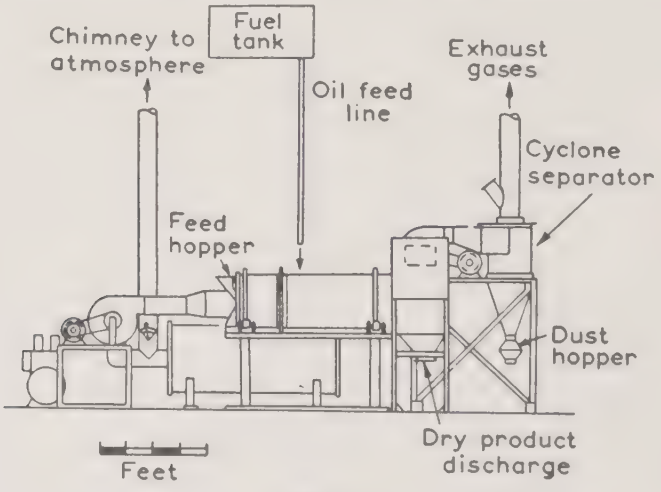


FIG. 1.

Research, Inveresk, is a pilot-plant installation with a drum 18 in. dia. × 7 ft. 6 in. long. Frond and stipe were minced to pass $\frac{3}{8}$ in. dia. before drying.

Stipe was satisfactorily dried in this dryer but the frond owing to its sticky nature adhered to the drum and caused irregular drying. A radioactive tracer technique clearly illustrated the irregular progress of the frond through the dryer[1].

2.2. *Pehrson Dual-Process Crop Dryer*

The next dryer tested was a Pehrson grass dryer at Kenton, Suffolk[2]. This full-sized unit (Fig. 2) with a throughput of 5

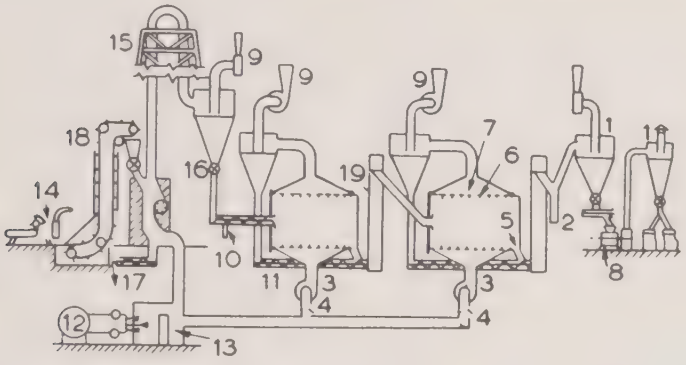


FIG. 2.

1. Bagging cyclones
2. Heavy fraction
3. Inlet hot-air fans
4. Secondary-air dampers
5. Main discharge
6. Tines
7. Rotating drum
8. Hammer mill
9. Exhaust fans
10. Sample point
11. Return conveyor
12. Oil
13. Furnace
14. Chaff cutter
15. Pneumatic tower
16. Air-tight sluice
17. Reject
18. Variable speed elevator
19. Bucket elevator

ton/hr. of grass, consists of pneumatic drying tower using furnace gases at 1800° F, followed by two rotary drying stages in which air at a lower temperature completed the drying.

Chaff-cut seaweed was fed into the base of flash drying tower where it was conveyed by the hot gases to a cyclone separator. The partly dried seaweed then passed through the two rotary dryers and emerged in a condition suitable for milling. Trouble was experienced in feeding the frond into the tower as the agglomerated particles were too heavy to be airborne and were discharged at the 'reject' port. Recycling of this frond in the hot gases resulted in a scorched product.

2.3. *Templewood Grass Dryer*

The third design tested was a double-pass through-circulation grass dryer (Fig. 3) with a throughput of one ton/hr. of grass when air at 300° F is used. The feed passes along the upper wire mesh belt and is returned by the lower conveyor, with the hot air passing through each layer in turn.

Tests on this unit demonstrated that chopped frond could be dried to give a satisfactory seaweed meal on a full-scale through

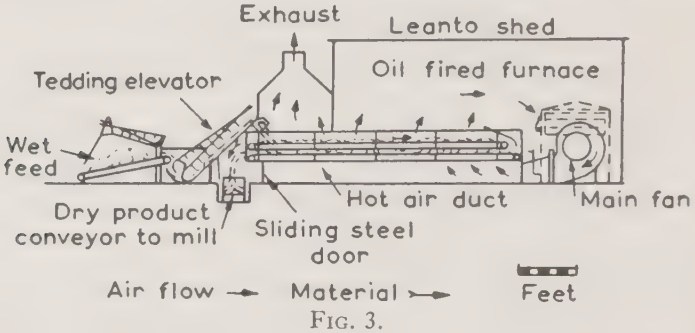


FIG. 3.

dryer, when the air temperature was maintained at 225° F. The Templewood dryer was not tested with stipe but numerous tests on a smaller dryer have verified that stipe can be readily dried by a through-draught of hot air.

2.4. *General Conclusions*

All three classes of dryer appeared to be technically capable of drying chopped or minced stipe in the freshly harvested state.

Fresh frond, owing to its sticky nature, gave trouble in both rotary and pneumatic dryers.

As frond was successfully dried in a through dryer, the method was selected for further investigation into the drying characteristics of seaweed.

3. LABORATORY DRYING TESTS

3.1. *Apparatus and Procedure*

The laboratory dryer (Fig. 4) contained the seaweed in a basket approximately 11 in. square × 9 in. deep. This was removed at regular intervals and weighed on a balance to a probable accuracy of 0·001 lb. After drying, the cooled seaweed was sampled and ground below 1 mm in a mill and its moisture content determined by oven drying for 5 hours at 220° F.

A bacon slicer was used to cut the fresh stipe as this gave a uni-

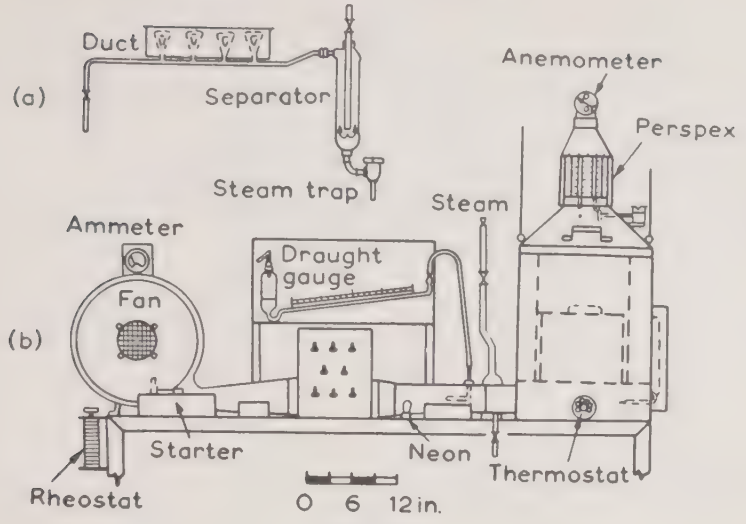


FIG. 4.

form slice thickness in a short time, whereas the frond was minced in a domestic mincer. Fig. 5 shows the variation of air flow, pressure drop and water content in a 3 in. bed of $\frac{1}{8}$ in. stipe slices during a typical run.

3.2. *Preliminary Tests*

The experimental technique was verified by the following tests[3]. Two tests on the same batch of seaweed showed that the inter-

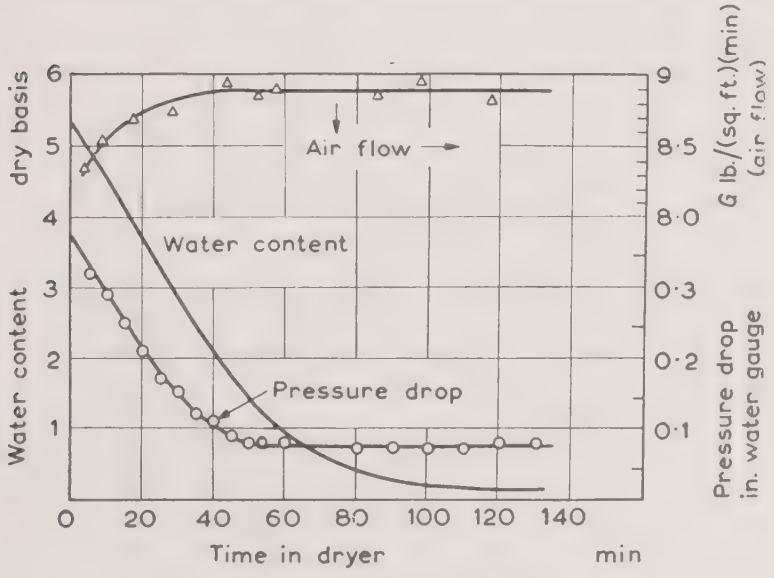


FIG. 5.

ruption of drying by the weighing operation caused negligible error, while a repeatability test showed that runs carried out under identical conditions gave drying curves which agreed closely.

It is known that in the drying of biological material the variation of initial water content makes direct comparison of tests difficult. Three tests were made with stipe of different initial water content but with the same bone-dry loading per unit area, which showed that samples of seaweed with the same bone-dry loading require the same time to dry (through the same water-content range) under the same drying conditions, irrespective of their original water content.

3.3 *Effect of Bed Depth*

As it is impracticable to measure bed depth directly it has been expressed as the dry loading (L) lb.B.D.S./sq.ft. bed area.

In cross-circulation drying, light loading of the wet material is generally necessary, since the air cannot penetrate readily to the lower regions of the bed and drying times become excessive. With through drying the air comes into contact with all parts of the bed and much heavier loadings may be practised.

Stipe—Stipe slices, $\frac{1}{8}$ in. thick, were dried at the average air conditions given in Table 1. When these runs were made, a fine speed control for the fan was not available, so that the average air flow for a run decreased as the bed depth was increased. The time required to dry the stipe from 5·0 to 0·15 water ratio is plotted against dry loading in Fig. 6, and the equation for this curve is given in Table 2.

The output of commercial dry seaweed (C.D.S.) is given by:

$$R=\frac{60L(1+F)}{\theta}$$ where F = the moisture content after time θ.

When this output is plotted against dry loading (Fig. 7), it is seen that the highest output is obtained with the deepest bed. The increase in output is most marked up to bed depths of about 3 in. ($L=1{\cdot}2$), and this loading was used in the subsequent stipe tests as it gave a convenient drying time. It should be noted from Fig. 5 that shrinkage of the bed is virtually complete at a water content of 1–1·5 lb./lb. B.D.S.

Frond—Frond particles are sticky and flexible in contrast to the uniformly-sized, rigid, non-adhesive stipe slices. The plot of dry-

Table 1. Average Drying Conditions for Runs

Species: *L. cloustoni* harvested at Oban.

Fig. No.	Part	Month	Size	D.B.T. °F	W.B.D. °F	*L*	*G*
5	Stipe	—	$\frac{1}{8}$ in.	156	75	1·33	—
6	Stipe	Apr.–May	$\frac{1}{8}$ in.	158	76	—	5·8
6	Frond	May	M.	156	75	—	7·1
8	Stipe	Apr.	—	155	72	1·23	6·6
9	Stipe	Mar.–May	$\frac{1}{8}$ in.	157	73	1·20	—
9	Frond	May	M.	156	75	0·75	—
10	Stipe	Apr.–June	$\frac{1}{8}$ in.	—	—	1·26	7·1
11	Stipe	Apr.	$\frac{1}{8}$ in.	—	1	1·28	7·5
12	Frond*	Apr.	M.	158	75	0·92	7·6

* *L. digitata* M = minced.

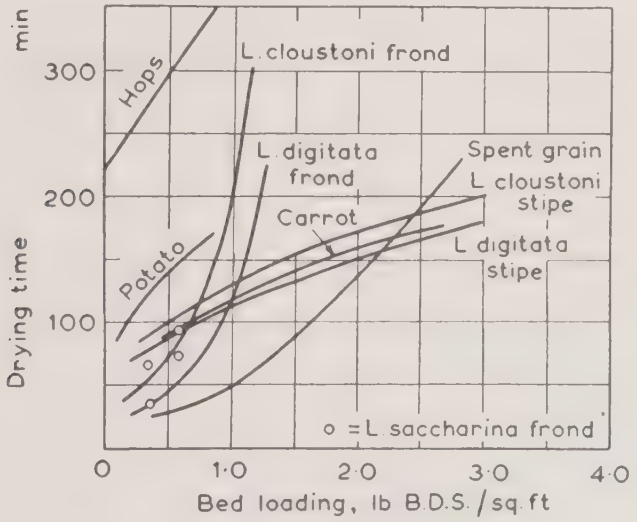


FIG. 6.

ing time versus bed loading for minced frond is included in Fig. 6, showing that the drying time increases rapidly for small increments of depth. When the output versus bed-loading curve is plotted (Fig. 7) a marked optimum loading is shown at 0·45 lb./sq. ft., beyond which the output rate falls away.

It is suggested that the disproportionate increase of drying time

Table 2. Equations for Drying Times

Species: *L. cloustoni* stipe (sliced) harvested at Oban.

Bed Depth	$\theta = 245\,[\log_{10}(L + 0{\cdot}94) + 0{\cdot}23]$
Air velocity	$\theta = \dfrac{G}{0{\cdot}0103G - 0{\cdot}02}$
Slice Thickness	$\theta = 410\,S^{0{\cdot}52}$
Temperature and Humidity	$\theta = \dfrac{9510}{\text{W.B.D.}}$

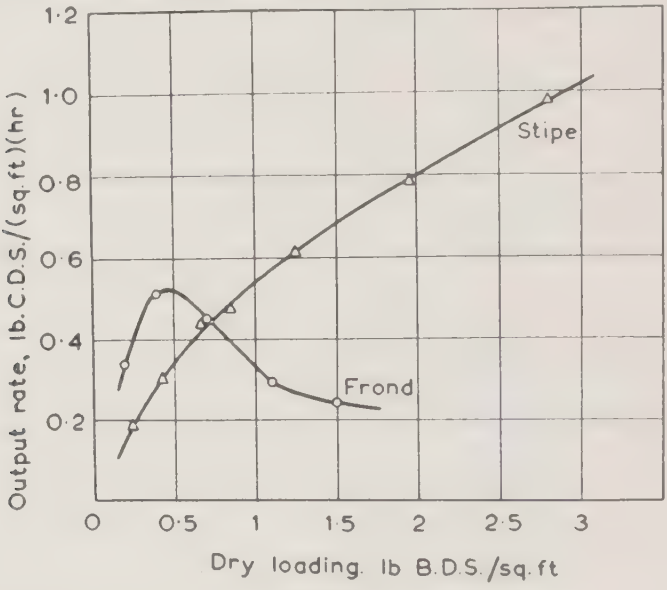


FIG. 7.

with increasing bed depth and the low optimum loading are the result of two factors: (1) stickiness of the frond, (2) lack of rigidity of the material.

The mucilage tends to fill the interstices between the individual particles, preventing the ready access of air, while the lack of rigidity of the fresh frond allows the lower strata to be compressed into a compact mass.

This is partly confirmed by tests on pre-dried frond[4] which was flexible but not sticky. Deep beds were easily dried but the curve was still slightly concave upwards probably caused by compression of the lower layers.

Comparison with other vegetable material—If the physical differences between stipe and frond control the relationship of drying

time to bed depth, then these differences should be general and should apply to vegetable material other than seaweed.

The physical characteristics in the two classes may be thus summarised:

Class I. Rigid or granular forming a porous bed. Only slight compression of lower layers. Examples: stipe or stalks.

Class II. Particles are soft, flexible, easily compressed. Bulk density increases markedly with increasing bed depth. Examples: frond or leaves.

Published through-drying tests for potato, hops and carrot[5–7] and tests on brewers' spent grain carried out on the present dryer are plotted in Fig. 6, which is intended to illustrate the shape of the curves for each material. It shows that potato and carrot behave in a similar manner to stipe slices whereas the spent grain curve resembles those for frond. Hops give a straight line, possibly because they approximate to spheres which are the ideal shape for through-circulation drying.

Curves which are linear or concave downward (Class I) will show an output curve which rises smoothly with bed depth whereas curves concave upward (Class II) will exhibit an optimum point in the output curve.

3.4. *Particle Size*

Stipe—Fig. 8 is a log log plot of drying time vs. slice thickness

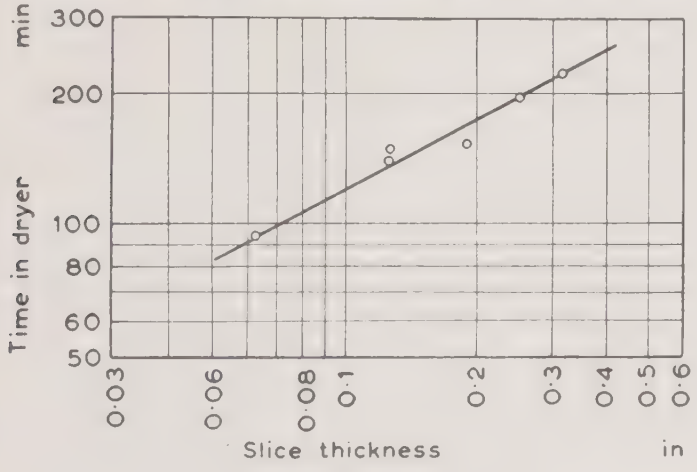


Fig. 8.

for stipe being dried between 5·0 and 0·15 water ratio. Slice thickness had relatively little effect on the drying rate in the initial stages but considerably shortened the times at the end of drying. The formula for this relationship is given in Table II.

Frond—A direct comparison between shredded and minced frond from the same batch of seaweed showed that the minced material dried in about 80% of the time required by the shreds. The bed of shredded material had a much lower static pressure drop to the passage of air.

3.5. *Air Velocity*

Stipe—In the air-flow tests, the fan speed was adjusted to a prearranged ammeter reading which was kept constant during a run.

As drying progressed, the seaweed bed contracted, its resistance decreased and the air flow increased until approximately midway through a test, when shrinkage ceased (Fig. 5). The average mass flow for a run was taken.

The drying time versus air flow plot is given in Fig. 9, the air flow being expressed as the mass rate per unit of bed area. It can be seen from the curve that there is little to be gained by using air flows greater than 8–9 lb./(sq.ft.)(min.).

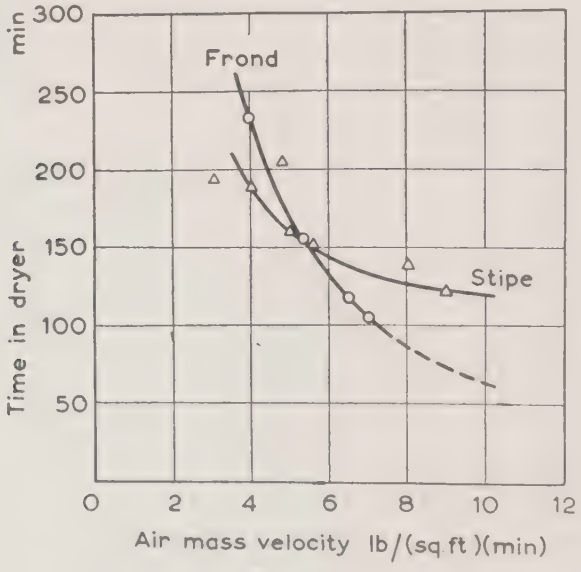


FIG. 9.

Frond—The corresponding curve for *L. cloustoni* frond shows the same general tendency as that for stipe.

3.6. *Temperature and Humidity*

In the following tests, the humidity of the inlet air has been expressed as the wet-bulb depression (W.B.D.) in agreement with the reasons advanced by EDE and HALES[9].

The W.B.D. was altered either by heating room air to various temperatures or by injecting steam into heated air to give low W.B.D. at a constant D.B.T.

Stipe—When the drying rates at various water contents are plotted against the W.B.D. of the inlet air (Fig. 10) it was found that the relationship is essentially linear so that drying rates are proportional to the W.B.D. even at water ratios as low as 0·2 lb./lb. B.D.S. Drying rates are independent of D.B.T. in the range tested, at least until the material is nearly dry. Drying times will also be inversely proportional to the W.B.D. (see Table 2).

Drying runs can now be correlated by a W.B.D. evaporation coefficient similar to that proposed by EDE and HALES[9] for cross-circulation drying. The standard drying curve is plotted for a W.B.D. of 1° F in Fig. 11. Drying times for other conditions can be calculated by dividing the time taken from Fig. 11 by the W.B.D.

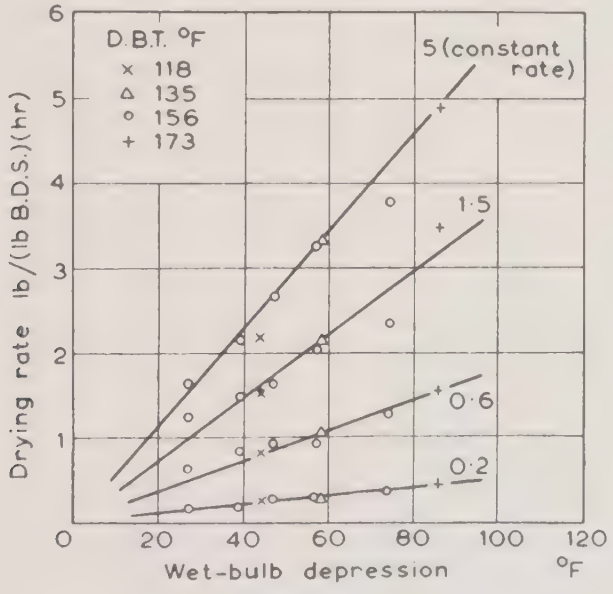


FIG. 10.

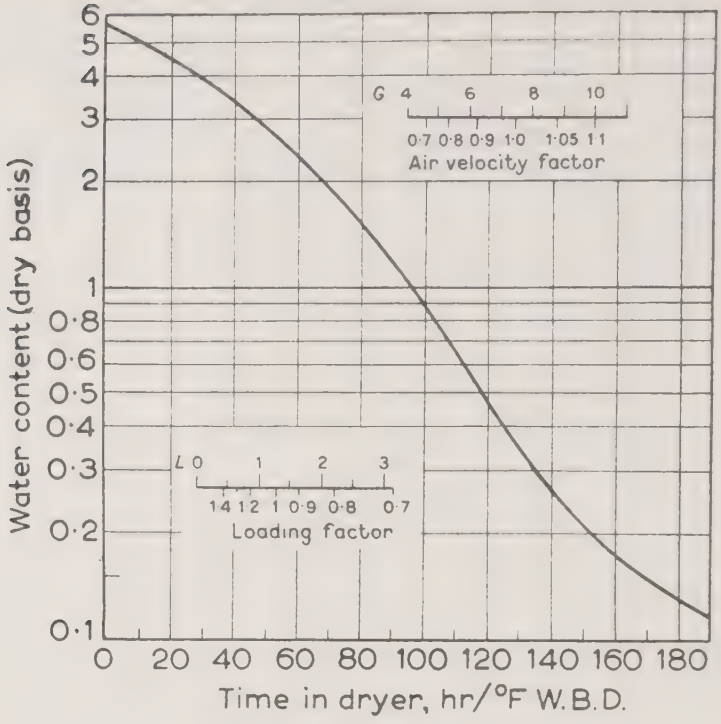


FIG. 11.

of the air and correcting for different air speeds and loadings by the appropriate factors given.

Frond—Frond beds can similarly be correlated by a W.B.D. coefficient.

Both stipe and frond beds show signs of scorching when exposed to air of about 225° F D.B.T. for a prolonged period.

3.7. *Agitation*

Stipe—One disadvantage of static bed through drying is that after the bottom layer is dry it is subjected to the full inlet-air temperature until the rest of the bed is dry. This can be overcome by reversing the direction of the air flow or by agitation of the bed. In one such test, a bed of stipe slices stirred every 10 minutes dried in 86% of the time required by a static bed.

Frond—During drying, a bed of minced frond shrinks from the basket sides allowing air to short-circuit the bed. Reversal of air flow is largely ineffective here as the air will take the line of least resistance. If the bed is mixed, larger masses containing knots of wet seaweed are broken up and shrinkage gaps are closed.

The drying times between the same limits of water content for different degrees of agitation are not known until the end of the run and consequently the agitations cannot be spaced out at equal time intervals between the initial and final water-content limits. If a run is carried out with, say, three agitations at twenty-minute intervals, and the final water content is reached five minutes after the last agitation, then the operation is strictly a four-stage process but the number of 'effective' states would probably be nearer 3·25. Although this concept of an effective stage is not ideal it attempts to proportion out the relative effect of mixings and enables comparisons to be drawn. An effective stage is defined as the 'agitated' drying time (between specified limits) divided by the time interval between stirrings.

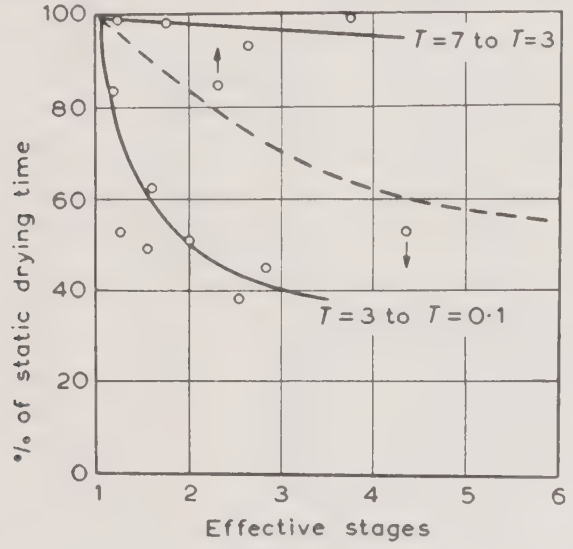


Fig. 12.

Fig. 12 indicates that the effect of agitation diminishes after 3 or 4 stages (i.e. 2 or 3 agitations) and also that mixing has a much greater effect in the later stages of drying.

3.8. *Miscellaneous Tests*

Other variables which have been studied included the temperature of a stipe slice during drying, comparisons between the species *L. cloustoni*, *L. digitata* and *L. saccharina*[8], and the seasonal variation of drying time for *L. cloustoni* frond. In the latter tests it was found that the drying time varied three- or four-fold depending on the month of harvesting[4].

Correlations of static pressure drop of the air passing through beds of seaweed have also been reported[3, 4].

4. THE DESIGN OF A CONTINUOUS THROUGH-CIRCULATION DRYER

The following examples illustrate how data from a laboratory batch dryer can be applied to the design of double-stage continuous dryers for seaweed and to the investigation of quantitative effects of operational changes on output.

Some of the possible arrangements of through dryers are given in Fig. 13.

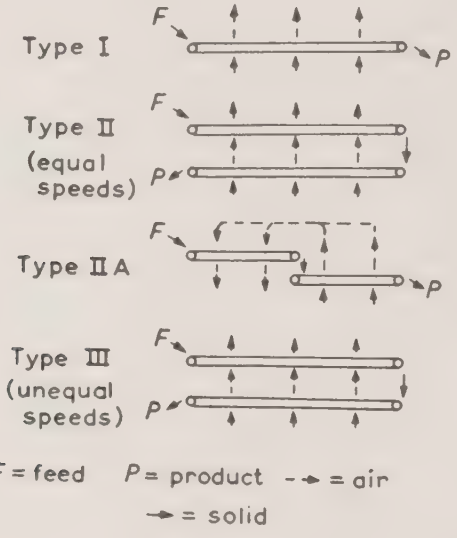


FIG. 13.

4.1. *Single-Stage Conveyor Dryer*

Example 1.—A continuous through-circulation dryer 60 ft. long × 6 ft. wide, is to be designed to dry *L. cloustoni* stipe slices ($\frac{1}{8}$th in. thick) from 5·5 to 0·15 water ratio with an air flow of 7·5 lb./(sq.ft.)(min.). Atmospheric air at 50° F D.B.T., 49° F W.B.T., is to be heated to 220° F D.B.T. and 95° F W.B.T. If the seaweed loading on the belt is 3 lb. B.D.S./sq.ft., determine (*a*) drying time, (*b*) output of C.D.S./hr., (*c*) evaporation rate and (*d*) specific evaporation. Total heat of air at 49° F W.B.T. = 12 B.Th.U./lb. and at 95° F W.B.T. = 54·9 B.Th.U./lb. from tables by MACEY[10].

(*a*) From the basic drying-time curve for *L. cloustoni* stipe slices (Fig. 11).

$\theta 5 - 0{\cdot}15 = 170$ hr./°F W.B.D.

Loading factor for *L*, 3·0 lb. B.D.S./sq.ft. = 0·72

Air velocity factor for *G*, 7·5 lb./(sq.ft.)(min.) = 1·0

Hence for 125°F W.B.D., $L = 3{\cdot}0$ and $G = 7{\cdot}5$

$$\text{drying time} = \frac{170}{1{\cdot}0 \times 0{\cdot}72 \times 125} = 1{\cdot}89 \text{ hr.}$$

(*b*) $$\text{Output} = \frac{360 \times 3 \times 1{\cdot}15}{1{\cdot}89} = 656 \text{ lb. C.D.S./hr.}$$

(*c*) $$\text{Evaporation} = \frac{656 \times 5{\cdot}35}{1{\cdot}15} = 3060 \text{ lb./hr.}$$

(*d*) $$\text{Heating load} = 7{\cdot}5 \times 60(54{\cdot}9 - 12) \times 360$$
$$= 6{,}950{,}000 \text{ B.Th.U./hr.}$$

Hence specific evaporation $= \frac{6{,}950{,}000}{3060}$

$= 2270$ B.Th.U./lb. of water evaporated.

This example represents the simplest design of through dryer (Type I of Fig. 13) and it assumed that there is no 'scale up' factor involved in the calculations for the full-sized dryer, and that there are no heat losses. Practical values of specific evaporation must also include the thermal efficiency of the furnace or air heater. This layout of dryer has a high output because the seaweed bed is supplied with hot dry air at all stages of drying, but has a correspondingly poor heat economy.

4.2. *Double-Stage Conveyor Dryer*

In this design, the 60 ft. belt is replaced by two superimposed 30 ft. belts, the upper one discharging on to the lower (Fig. 13, Type II). Hot air leaves the dried seaweed at the discharge end and passes through the fresh seaweed on the upper belt, giving improved thermal efficiency but lower output.

Example 2.—A two-stage through dryer with belts 30 ft. × 6 ft. is to dry *L. cloustoni* $\frac{1}{8}$ in. stipe slices under the same conditions as Example 1. Both belts are to run at the same speed with a loading of 3·0 lb./sq.ft. Calculate (*a*) drying time, (*b*) water ratio at the change-over, (*c*) product rate, (*d*) evaporation rate and (*e*) specific evaporation.

The problem is to determine the total drying time to make the times in each stage equal. A complication is that the humidity of the air entering the upper belt is not constant along the length of the dryer. To obviate the possibly tedious calculations of trial and error methods, the following graphical design method has been devised[11].

1. From the basic drying curve calculate the water content/time curve of the lower belt for the given conditions and plot it with a reversed time scale, i.e. with zero time at 0·15 water ratio.
2. Select suitable equal time-increments and interpolate the terminal water ratios for each increment from the curve.
3. Calculate the water picked up by the air passing through each increment of the bed (by using equation below).

4. Add the water pick-up to the inlet-air humidity to give the humidity of the air leaving each section of the lower belt. Assume a constant W.B.T. for the hot air and obtain the D.B.T. and hence the W.B.D. from a psychrometric chart.
5. From the basic drying curve and the appropriate W.B.D. compute the water content of the seaweed on the top belt as it reaches the end of each successive time-increment.
6. Plot the water ratio/time curve for the upper belt until it intersects the corresponding curve for the lower belt. This intersection gives the water content at the turnover and the drying time on each belt (which are equal in this case).

The water pick-up in lb. water/lb. dry air is given by: $L(T_0 - T_i)/G\theta$ where T_0 and T_i = terminal water ratios for each increment.

Table 3 gives the tabulated calculations, and Fig. 14 shows the graphical solution to the problem.

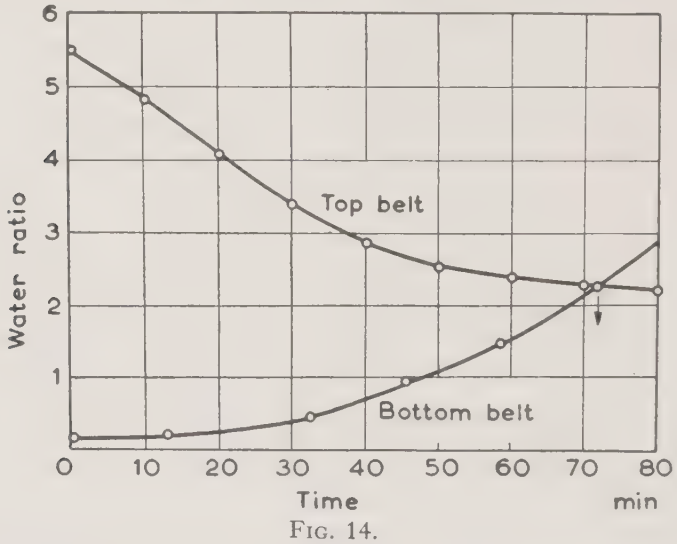


FIG. 14.

(*a*) and (*b*). By interpolation from Fig. 14, the drying time on each belt is 72 min. and the water ratio at the turnover is 2·3.

(*c*) Product rate $= \dfrac{180 \times 3 \times 1{\cdot}15 \times 60}{72} = 517$ lb.C.D.S./hr.

(*d*) Evaporation rate $= \dfrac{180 \times 3 \times 5{\cdot}35 \times 60}{72} = 2410$ lb./hr.

(*e*) Heating load $= 450\ (54{\cdot}9 - 12) \times 180$
$= 3{,}480{,}000$ B.Th.U./hr.

Specific evaporation $= \dfrac{3{,}480{,}000}{2410} = 1440$ B.Th.U./lb.

It can be seen that the heating load is halved by superimposing the belts, and that the air is much more effectively used than in a

Table 3

Increment, min.	Terminal water ratios	ΔT	Water pick-up, lb./lb.	Exhaust air from lower belt Humidity, lb./lb.	D.B.T. °F	W.B.D. °F
0–10	0·15–0·20	0·05	0·0020	0·0092	211	116
10–20	0·20–0·28	0·08	0·0032	0·0104	206	111
20–30	0·28–0·40	0·12	0·0048	0·0120	200	105
30–40	0·40–0·65	0·25	0·0100	0·0172	177	82
40–50	0·65–1·05	0·40	0·0160	0·0232	151	56
50–60	1·05–1·60	0·55	0·0220	0·0292	125	30
60–70	1·60–2·20	0·60	0·0240	0·0312	116	21
70–80	2·20–2·90	0·70	0·0280	0·0352	99	4

single-pass dryer which must discharge relatively dry air when the bed is nearly dry.

An alternative way of achieving the same result is to arrange the belts as in Fig. 13, Type IIA. The graphical method can be adapted to the design of this type of dryer, although the tandem layout would be less compact.

ACKNOWLEDGEMENTS

One of us (R.G.G.) is indebted to the Institute of Seaweed Research for a 3-year post-graduate research scholarship.

REFERENCES

1. Gardner, R. G., Mitchell, T. J., and Scott, R., *Chem. and Ind.*, p. 448 (1952).
2. Gardner, R. G., Scottish Seaweed Research Association, Report No. 145 (1951).
3. Gardner, R. G. and Mitchell, T. J., *J. Sci. Fd. Agric.*, **4**, 113 (1953).
4. Gardner, R. G., and Mitchell, T. J., *J. Sci. Fd. Agric.*, **4**, 237 (1953).
5. Brown, A. H. and Van Arsdel, W. B., Drying Rate Nomographs. III. White Potato Strips—Vertical Air Flow. U.S. Dept. Agric. A1C—31—III, 1944 (Reissued 1951).
6. Burgess, A. H., *Wallerstein Lab. Commun.*, **14**, No. 45, 111 (1951).
7. Ede, A. J., 'Dehydration', U. K. Progress Reports. Section X, Part 2. D.S.I.R. and Min. of Food, 1953 (London, H.M.S.O.).
8. Gardner, R. G. and Mitchell, T. J., *J. Sci. Fd. Agric.*, **4**, 364 (1953).
9. Ede, A. J. and Hales, K. C., 'The Physics of Drying in Heated Air with Particular Reference to Fruit and Vegetables', *D.S.I.R. Food Invest. Spec. Rept.* No. 53 (1948).
10. Macey, H. H., *Drying in the Heavy Clay Industries*, Min. of Works, 1950 (London, H.M.S.O.).
11. Gardner, R. G., and Mitchell, T. J., *J. Sci. Fd. Agric.*, **5**, 481 (1954).

DISCUSSION

Mr. POWLING had noticed a flattening out of the curves of the rate of evaporation at a point corresponding to a water content of 10–15%. He wondered if this might be due to case hardening. He also asked Dr. GARDNER if he had tried to disintegrate the stipes further before the material entered the last stage in his two-stage dryer.

Dr. GARDNER answered that the first point had not been fully investigated but in his opinion no serious instance of case hardening occurred. Size reduction of the stipes during drying did not seem to increase the rate of evaporation and in addition, the disintegration itself would be quite a problem. At a certain water content the slices turned rubbery to an extent that made hammer mills useless.

Mr. HAUG asked Dr. GARDNER if any investigations had been carried out to determine the effect of the drying conditions on the viscosity of the alginic acid prepared from this artificially dried material.

Dr. GARDNER said that no such investigations had been carried out yet. The drying experiments were first carried out with stipes and according to Dr. BLACK the viscosity of alginic acid from this material would be quite low at any rate. Accordingly, the drying conditions were not expected to affect the viscosity. When the optimum drying conditions were established their influence on the viscosity might be tested.

Mr. HAUG pointed out that according to Dr. BLACK the viscosity of alginic acid from *Laminaria digitata* dropped rapidly even when drying at room temperature. The experience of Mr. HAUG was that rapid drying at 60 to 80° C hardly affected the viscosity at all.

Dr. GARDNER stated that he had been concerned with the extraction of laminarin, and the weed had to be dried fairly rapidly to avoid decomposition of this compound. A compromise had to be made between the quality of the product and the feasibility of the method of preparation.

Mr. FLOOD wanted to know at what time of the year the weed had been harvested and under what conditions the raw material had been stored before the experiments were carried out.

Dr. GARDNER replied that in general only a few hours elapsed between harvesting and drying. One experiment with fronds had been carried out when part of the material was dried immediately, another part stored for one day and the rest stored for 3 days. He found that the drying time increased on standing (57 minutes immediately, 64 minutes after one day and 79 minutes after 3 days of storage). Regarding the time of the year he had not observed any relation between season and drying time of the stipes, but with the fronds marked influence of the season was found. When the drying time was plotted against the month of the year, the maximum drying time of *Laminaria cloustoni* fronds was clearly shown to occur in June.

These experiments had been carried out under standard conditions, the material being dried between standard water contents. In addition to the differences in standard drying times, the wide variation in the initial total water content had to be taken into account.

Mr. FLOOD mentioned that according to some of his experiments storage of fresh *Laminaria Cloustoni* for even one day, particularly in sacks, might lead to considerable decomposition. A strong odour of nitrogen oxide had been observed on several occasions when such weed was immersed in hydrochloric acid. In particular the fronds seemed to be liable to decay.

Dr. GARDNER found that the rate of decomposition depended on the locality from which the weed was taken. This probably had something to do with the pollution of the sea water.

Mr. JENSEN wanted to know if the increased drying time observed in June had anything to do with the mannitol content of the algae.

Dr. GARDNER regretted that this point had not been fully investigated and that he did not know the answer.

Mr. MCDOWELL asked Dr. GARDNER if he had ever tried to rinse the weed with fresh water before drying.

Dr. GARDNER stated that in all cases previously discussed by him the weed had been dried directly from the natural state. He was afraid that treatment with fresh water would remove some of the valuable constituents. Some preliminary experiments on the influence of drying by fresh water washing seemed to indicate that this had only a slight effect on the drying time.

Mr. KRISTENSEN mentioned that according to his experience *Laminaria digitata* for alginate production ought to be dried within 6 hours after the harvesting. This was one of the factors determining the size of the dryer and he wanted to know what capacities Dr. GARDNER's dryers had.

Dr. GARDNER answered that he had carried out a typical calculation for a double belt dryer 30′ × 6′, air temperature 220° F, wet bulb temperature 95° F at 7·5 lb. of air per square foot per minute. The output of the dryer would be 517 lb. of commercially dry solid with a water ratio of 0·15. The evaporation rate corresponding to this was 2410 lb. per hour, the heating load would be 35 therms per hour and the specific evaporation 1440 B. Th. U. per lb. water evaporated.

Mr. TORGERSEN jr. asked Dr. GARDNER if he had tried to dry *Ascophyllum nodosum*, to which Dr. GARDNER answered that some of his colleagues had been working with *Ascophyllum* and *Fucus serratus*. As with *L. cloustoni* fronds, an optimum bed depth was found for this material.

Mr. GLYNN, Ireland, had noticed that the dried weed coming hot from the dryer was very difficult to grind, but this obstacle disappeared after cooling the material. He wanted to know if Dr. GARDNER had made similar observations.

Dr. GARDNER replied that he had always allowed the weed to cool before grinding, and in addition he was always drying to a water content of about 10 per cent. This might eliminate all the difficulties encountered in grinding.

EXPERIMENTS WITH SEAWEED MEALS AS SUPPLEMENTS TO RATIONS FOR CHICKS AND LAYING HENS

By J. Høie and Ø. Sandvik (Norway)

Read by J. Høie

The value of seaweed meals as supplements to chick and hen rations has been investigated among others by MacIntyre and Jenkins[3]. In these investigations no special effects of a 2·5% or 10% amount of *Ascophyllum* meal in the total feed of laying hens could be found on either the state of health, the weight of the hens the production of eggs, the strength of shells or the hatchability of the eggs. An addition of 2·5%, 5%, and 10% *Ascophyllum* meal to the chick feed had no noticeable effect, but one of 20% *Ascophyllum* meal resulted in poor development and a higher mortality rate.

In experiments at Reading (Black[1]), 10% of the total feed was replaced by *Laminaria* and *Ascophyllum* meals and given to R.I.R. pullets in full production without any noticeable effect on health or egg production. Twenty per cent seaweed meal plus mineral supplements had a disturbing effect on the hens' metabolism.

Romijn and Lokhorst[5] of the Netherlands included 1%, 5%, and 10% Algit *Ascophyllum* meal in the total feed of laying hens. The resulting higher iodine content apparently had no effect on the laying, but the moulting period seemed to be shorter for the hens getting the 1% Algit than for the control group and the other seaweed groups. It should be mentioned, however, that the group on 1% Algit was not set up until towards the end of the experiment, just before moulting was at its strongest in all groups, and the best hens in the original control group were chosen.

The high iodine content in the feed led to a large iodine content in the eggs, larger thyroid glands of irregular form, and other histological alterations.

PRESENT EXPERIMENTS

The Institute of Poultry and Fur Animals at the Agricultural College of Norway, Vollebekk, during 1951–54 carried out six experiments with growing chicks (148K, 186K, 235K, 237K, 239K, and 403K) and two with laying pullets (232H and 402H), all from the Institute's stock of White Leghorn, in order to investigate the value of different types of seaweed meal supplements in chick and hen rations. The total number of chicks in the chick experiments was 2356, and the total number of hens was 544. The types and the various compounds used in the experiments are listed in Table 1.

A basal diet + seaweed meal was compared to a basal diet only, and in most cases, also to a basal diet + commercial grass meal and/or yeast. According to the manufacturer the grass meal was produced from a very composite plant material harvested at several different stages of development. The chemical composition was: 92% dry matter, 7·7% ashes, 13·4% protein ($N \times 6{\cdot}25$), 26·5% fibre and 4·1% ether extract.

Experiments on Chicks

The chicks were on experimental conditions from the time they were 1 day old until they were 6 or 8 weeks, and they were all placed in cages with wire floors and had free access to dry mash and water.

In experiment 148K a basal diet including cod liver oil, yeast and grass meal supplemented with 3% seaweed meal[1a] was compared to a basal diet alone (Table 2). Two groups of 70 chicks (mixed sexes) were used.

The chicks in both groups grew satisfactorily, and no effect of the seaweed meal supplement could be noticed on the body weight, food consumption or health conditions during the 8 weeks.

Experiment 186K had 8 groups of 67 chicks (mixed sexes) and lasted until the chicks were 8 weeks old.

Basal diet with 2% yeast + cod liver oil (c. 750 I.U. A and 75 I.U. D_3 per gram) was compared with:

2. Basal diet + 2% cod liver oil + 5% one-year-old grass meal
3–5. Basal diet + 2% cod liver oil + 4% *Ascophyllum* meal[1a, 1b or 1c]

Table 1. Survey of the Seaweed Meals used in the Experiments

Seaweed species	When harvested	Composition of the meals, per cent										Reference to the report
		Dry matter	Ash	Mannitol	Alginic acid	Laminarin	Fucose	Protein	Ether extract	Crude fibre	Iodine	
Ascophyllum nodosum	Commercial	89·6	18·7	6·8	10·8	9·8	5·8	5·1	4·3	5·8	0·053	1 *a*
Ascophyllum nodosum	25/2–1951	89·3	20·2	4·8	11·5	7·4	3·5	7·0	3·1	5·2	0·087	1 *b*
Ascophyllum nodosum	22/3–1951	87·5	17·0	6·3	14·3	6·2	3·6	5·7	3·3	6·0	0·054	1 *c*
Ascophyllum nodosum	July–August 1952	—	—	—	—	—	—	—	—	—	—	1 *d*
Ascophyllum nodosum	July 1953	—	—	—	—	—	—	—	—	—	—	1 *e*
Alaria esculenta	26–28/5–1951	86·4	25·6	6·8	20·7	4·1	1·7	9·1	1·0	6·8	0·072	2
1/3 *Laminaria hyperborea* 1/3 *L. saccarina* 1/3 *Alaria esculenta*	4–6/9–1952	90·6	20·8	14·4	23·4	14·5	—	9·4	0·71	11·3	0·246	3
Laminaria hyperborea	9–10/5–1953 young lamina	91·2	29·2	5·0	—	2·0	—	12·2	1·0	9·4	0·370	4 *a*
Laminaria hyperborea	20/9–1953	93·8	19·5	15·8	—	19·5	—	5·9	0·8	9·3	0·440	4 *b*

6. Basal diet + 2% cod liver oil + 4% *Alaria* meal[2]
7. Basal diet + 4% *Ascophyllum* meal [1c]
8. Basal diet + 3·5% (gross meal + 3·5% *Ascophyllum* meal[1c].

Table 2. The Composition of the Basal Mash

	Chick experiment, No.			Pullet experiment, No.			
				232H			
	148K	186K	235K 237K 239K 403K	18/12–52–30/1–53	31/1–53–31/3–53	1/4–53–31/7–53	402H
Ground yellow corn %	32·0	35·0	35·0	25·0	24·0	23·0	31·0
Ground barley ,,	14·0	—	5·0	21·0	21·0	20·0	7·5
Ground oats ,,	7·0	9·0	12·0	22·0	22·0	19·0	10·0
Ground wheat ,,	8·0	25·0	19·0	—	—	—	10·0
Wheat bran ,,	6·0	7·0	7·0	12·0	11·5	11·5	15·0
Soyabean oil meal ,,	18·0	8·0	8·0	—	3·0	6·0	5·0
Peanut oil meal ,,	—	—	—	—	—	—	5·0
Linseed oil meal ,,	—	2·0	2·0	—	—	—	2·0
Herring meal ,,	2·0	8·7	8·7	14·5	13·0	15·0	8·0
Grass meal ,,	5·6	—	—	—	—	—	—
Feed yeast ,,	2·0	2·0	—	—	—	—	—
Cod liver oil ,,	2·0	—	—	1·0	1·0	1·0	1·0
Ground limestone ,,	1·1	1·0	1·0	1·0	1·0	1·0	2·0
Dicalcium phosphate ,,	2·0	2·0	2·0	3·0	3·0	3·0	3·0
Common salt ,,	0·3	0·3	0·3	0·5	0·5	0·5	0·5
Total %	100	100	100	100	100	100	100
Crude protein %	18·5	19·6	19·4	17·8	19·2	21·8	19·0
Ca ,,	1·08	1·20	1·19	1·57	1·54	1·60	1·79
P ,,	0·74	0·88	0·89	1·12	1·10	1·14	1·20
Vitamin A and Carotene, calculated I.U./kg	16500	1680	1680	8720	8670	8620	9013
Riboflavin, calculated mg/kg	3·4	2·5	1·7	1·79	1·70	1·74	1·85

All the groups had satisfactory body weight (Fig. 1) and good health, no significant difference was found in the weight increase of the different groups. Since none of the groups was on basal diet only, it was impossible to determine whether or not the basal diet

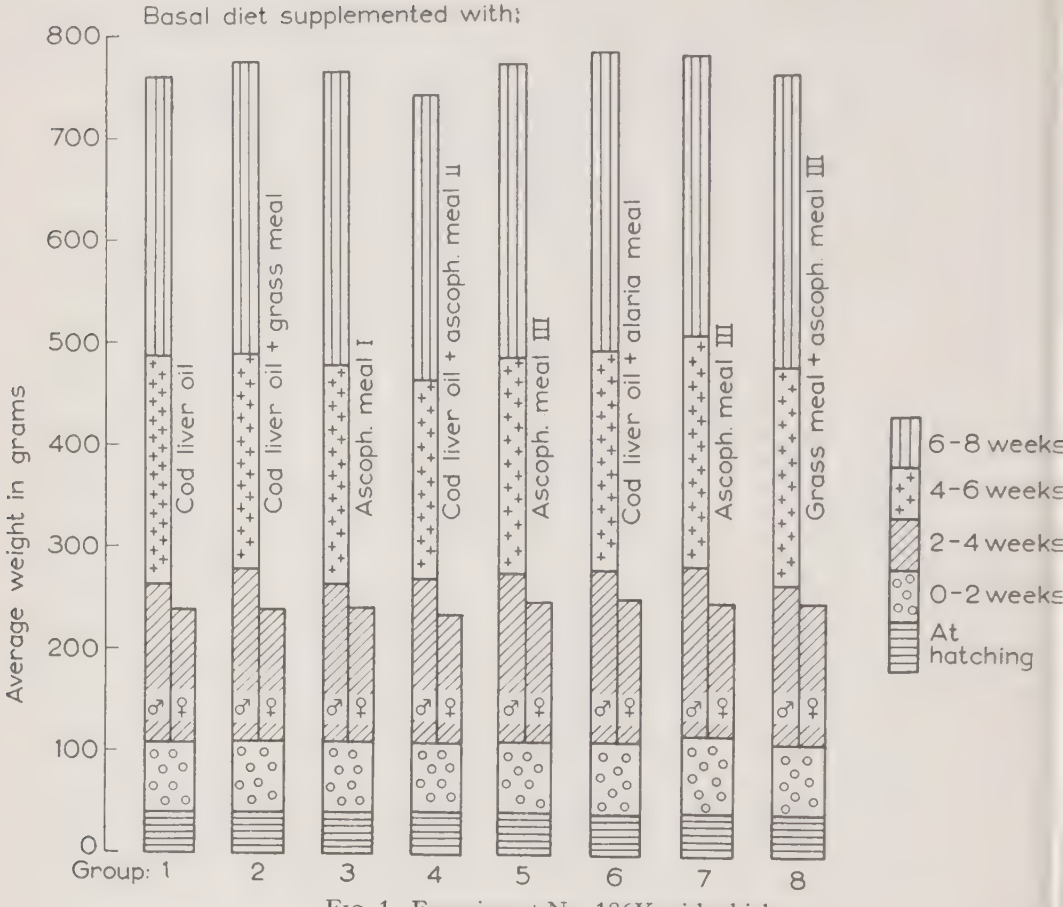


FIG. 1. Experiment No. 186K with chicks.

itself contained enough carotene and vitamin D to cover the chicks' requirements.

In experiments 235K, 237K, 239K and 403K the basal diet was not supplemented by yeast, grass meal or cod liver oil and had a very low content of riboflavin. The chicks on basal diet grew poorly and many had leg weakness. Supplements of 7% *Ascophyllum* meal[1d], 7% *Laminaria* meal[3] or 5% *Laminaria* meal [4a, 4b] in part resulted in a considerable increase in body weight as well as in better health condition. The same was true for grass meal and feed yeast (Table 3 and Fig. 2, 3 and 4).

In one experiment, 403K, a 5% supplement of meal from young lamina of Laminaria hyperborea harvested in May[4a] gave an extra increase in body weight of 30% during the 0–6 week period,

Table 3. Diets and Gain in Weight in Experiments 235K, 237K, 239K and 403K

Experiment No.	No. of chicks per group	Group No.	Composition of diets, per cent					Gain, per cent of group No. 1	
			Basal	Cod liver oil emulsion	Grass meal	Feed yeast	Seaweed meal	Males 0–8 weeks	Females 0–8 weeks
235K	30	1	100·0	—	—	—	(1)	100·0	100·0
		2	93·0	—	7·0	—	—	146·1	138·9
		3	93·0	—	—	—	7·0	168·7	130·0
		4	91·0	—	7·0	2·0	—	150·3	137·3
		5	91·0	—	—	2·0	7·0	152·1	129·4
		6	92·0	1·0	—	—	7·0	152·1	163·1
		7	90·0	1·0	—	2·0	7·0	215·1	218·4
		8	90·0	1·0	7·0	2·0	—	222·9	208·5
237K	60	1	100·0	—	—	—	(2) —	100·0	
		2	93·0	—	7·0	—	—	121·2	
		3	93·0	—	—	—	7·0	116·4	
		4	91·0	—	7·0	2·0	—	126·3	
		5	91·0	—	—	2·0	7·0	128·2	
		6	92·0	1·0	—	—	7·0	122·2	
		7	90·0	1·0	—	2·0	7·0	124·5	
		8	90·0	1·0	7·0	2·0	—	130·9	
239K	70	1	100·0	—	—	—	(1) —	100·0	
		2	93·0	—	7·0	—	—	130·3	
		3	93·0	—	—	—	7·0	125·4	
		4	91·0	—	7·0	2·0	—	139·3	
		5	91·0	—	—	2·0	7·0	139·4	
		6	92·0	1·0	—	—	7·0	125·9	
		7	90·0	1·0	—	2·0	7·0	147·2	
		8	98·0	2·0	—	—	—	100·1	
403K	50	1	100·0	—	—	—	—	(5) 100·0	
		2	95·0	—	5·0	—	—	118·4	
		3	95·0	—	—	—	5·0(3)	109·8	
		4	95·0	—	—	—	5·0(4)	130·1	
		5	98·0	—	—	2·0	—	142·5	
		6	93·0	—	5·0	2·0	—	137·0	
		7	93·0	—	—	2·0	5·0(3)	144·9	
		8	93·0	—	—	2·0	5·0(4)	140·8	

(1) *Ascophyllum* meal harvested July–August 1952 (a)
(2) Meal from ⅓ *Laminaria hyperborea*, ⅓ *L. saccharina*, ⅓ *Alaria esculenta* harvested 4–6/9 1952 (3)
(3) *Laminaria hyperborea* harvested 9–10/5 1953 (4a).
(4) *Laminaria hyperborea* harvested 20/9 1953 (4b).
(5) 0–6 weeks.

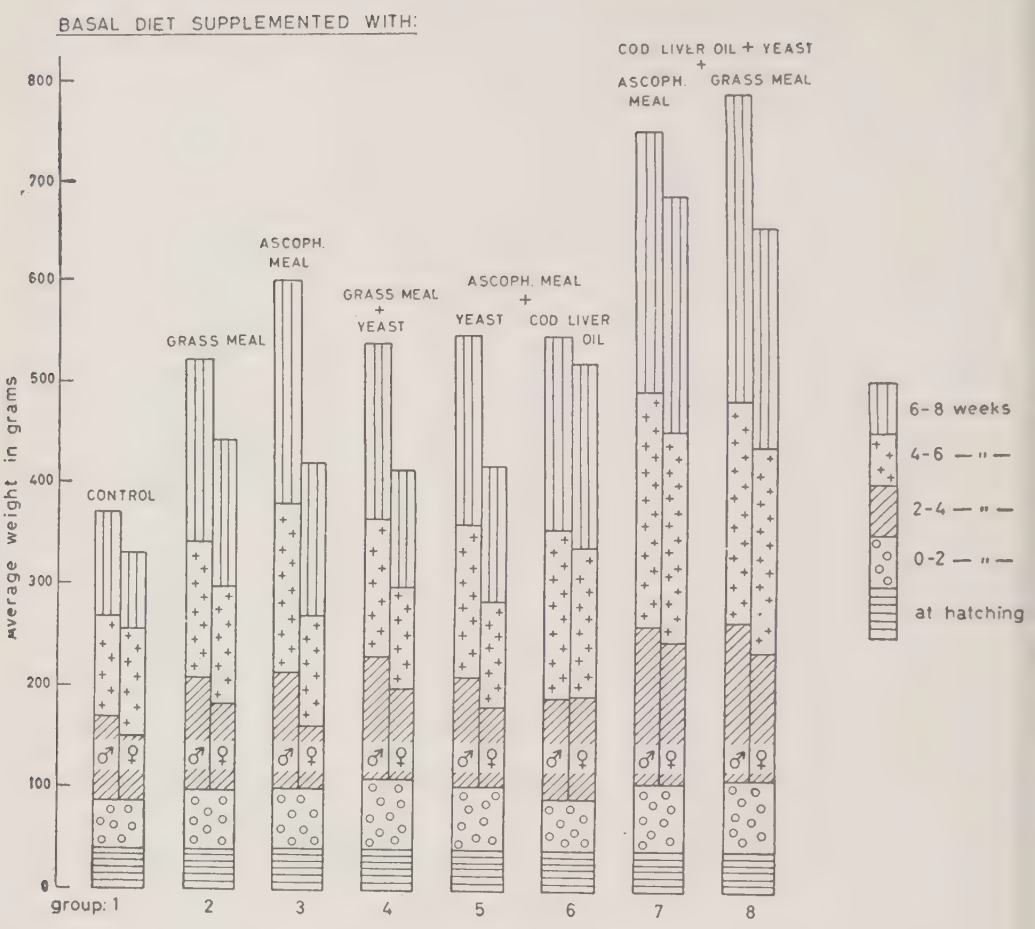


FIG. 2. Experiment No. 235K with chicks.

while 5% of a meal from the same species of alga harvested at the same place in September[4b] gave an extra increase of only 9·8% (Table 3 and Fig. 5).

Experiment on Laying Pullets

In the two experiments on hens each pullet received 40 g of whole corn (maize) daily, and had free access to a basal diet supplemented with *Ascophyllum* or *Laminaria* or grass meal. The control groups received basal diet only. In the first experiment (232H), which lasted 7·5 months from 18 December 1952 to 31 July 1953, all the groups were given 25 g of skim milk per hen per day for 3·5

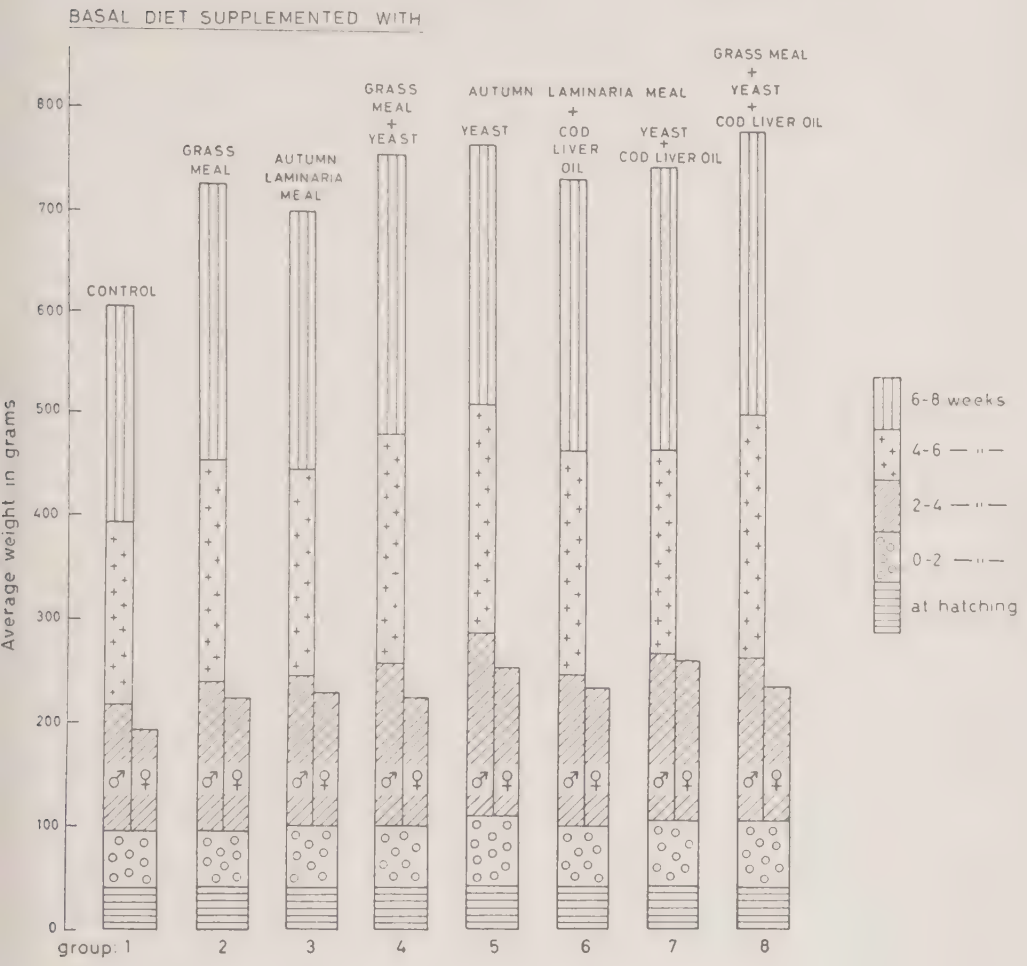


FIG. 3. Experiment No. 237K with chicks.

months from 27 February to 6 June 1953. In the second experiment (402H) 3 groups were given 50 g of skim milk per hen per day while 5 groups received no milk.

The first experiment (232H) was divided into 8 groups of 32 pullets which received the following diets:

Group 1: Basal diet during the entire period.

Group 2: Basal diet to April 25, then an 8% supplement of grass meal.

Group 3: Basal diet to April 25, then an 8% supplement of *Ascophyllum* meal.

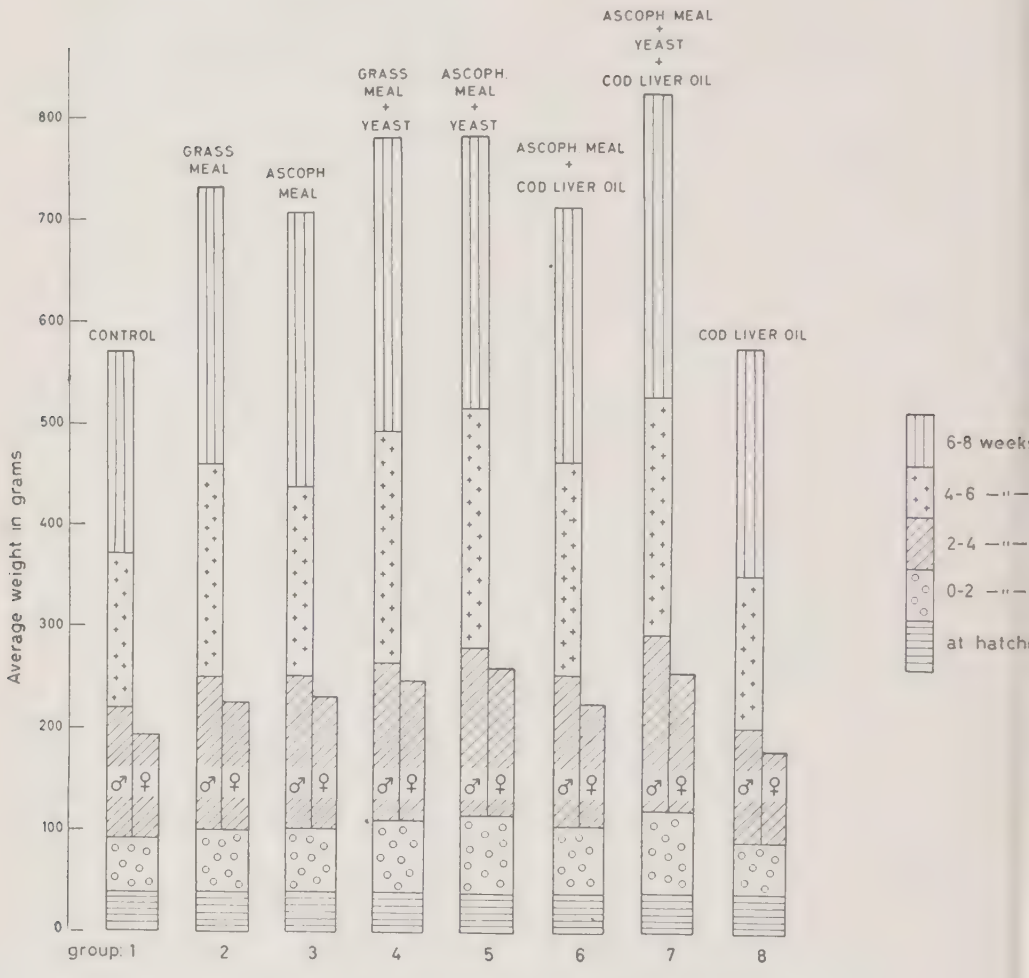


FIG. 4. Experiment No. 239K with chicks.

Group 4: Basal diet the entire period; the hens were on a built-up litter.

Group 5: Basal diet until April 25, then an 8% supplement of *Laminaria* meal.

Group 6: Basal diet + 8% grass meal during the entire period.

Group 7: Basal diet + 8% *Ascophyllum* meal[1d] during the entire period.

Group 8: Basal diet + 8% *Laminaria* meal[3] during the entire period.

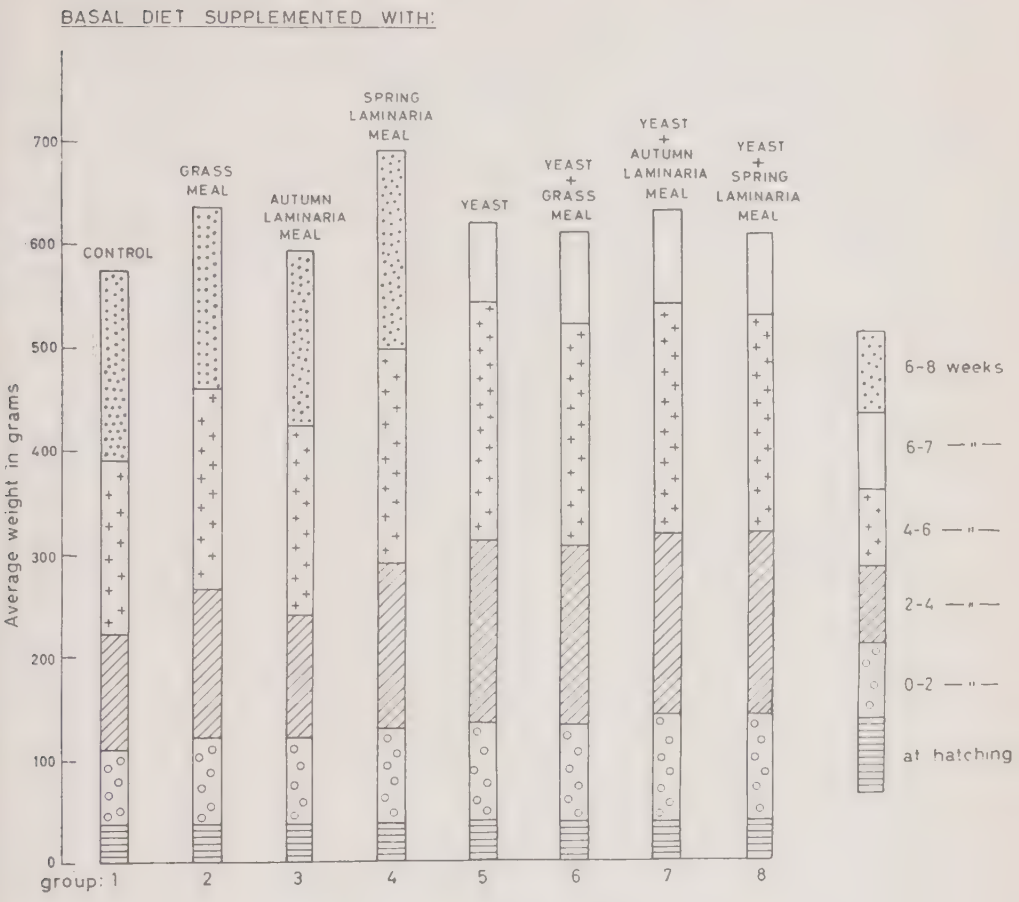


FIG. 5. Experiment No. 403K with chicks.

In the first part of this experiment the nourishment of the groups receiving basal diet was somewhat deficient (Table 2), and the laying per cent went down to 20–30%. There was a tendency towards better laying in the groups which received an 8% supplement of *Ascophyllum*[1d], *Laminaria* or grass meals, as there was in Group 4 on built-up litter (Table 4 and Fig. 6). However, the production as a whole was very low and none of the groups had satisfactory laying. When the basal diet was later made richer in protein, and all the groups received an addition of 25 g skim milk per hen per day, the laying went up sharply and the difference between the groups became even smaller.

The results of the hatching experiments are given in Table 4

Table 4. Egg-laying and hatching results in experiments 232H and 402H

	Group no.	Diets		Percentage hen-day production		Number of eggs incubated		Percent hatch of fertile eggs	
		18/12–24/4	25/4–31/7	18/12–24/4	25/4–31/7	18/12–24/4	25/4–31/7	18/12–24/4	25/4–31/7
Experiment 232H	1	Basal	Basal	47·9	70·6	549	300	12·7	24·2
	2	Basal	Basal + Grass meal	46·6	73·1	557	300	11·4	43·6
	3	Basal	Basal + Seaweed meal (1*d*)	48·6	67·6	560	299	12·0	28·5
	4	Basal, built up litter	Basal, built-up litter	51·8	64·7	570	304	15·5	41·5
	5	Basal	Basal + Seaweed meal (3)	45·4	65·2	526	300	10·7	28·3
	6	Basal + Grass meal	Basal + Grass meal	55·2	63·5	589	296	32·7	44·7
	7	Basal + Seaweed meal (1*d*)	Basal + Seaweed meal (1*d*)	56·1	67·1	577	300	11·1	26·3
	8	Basal + Seaweed meal (3)	Basal + Seaweed meal (3)	53·4	72·2	558	296	17·7	30·2
Experiment 402H	1	Basal		75·7		400		68·0	
	2	Basal + Grass meal		76·3		550		85·8	
	3	Basal + Seaweed meal (4*a*)		74·0		450		80·2	
	4	Basal + Seaweed meal (1*e*)		76·4		400		79·6	
	5	Basal + Grass meal + Seaweed meal (1*e*)		78·1		550		76·1	
	6	As 1 + skim milk		76·3		400		86·5	
	7	As 2 + skim milk		77·8		550		82·5	
	8	As 3 + skim milk		76·6		450		75·0	
	9	As 4 + skim milk		81·8		400		87·2	
	10	As 5 + skim milk		74·0		550		84·1	

and Fig. 6. The supplement of grass meal very clearly resulted in better hatching, especially in the first part of the experiment. *Laminaria* meal[3] had only a slight effect and the *Ascophyllum* meal[1d] had no effect at all. During the spring and summer, eggs

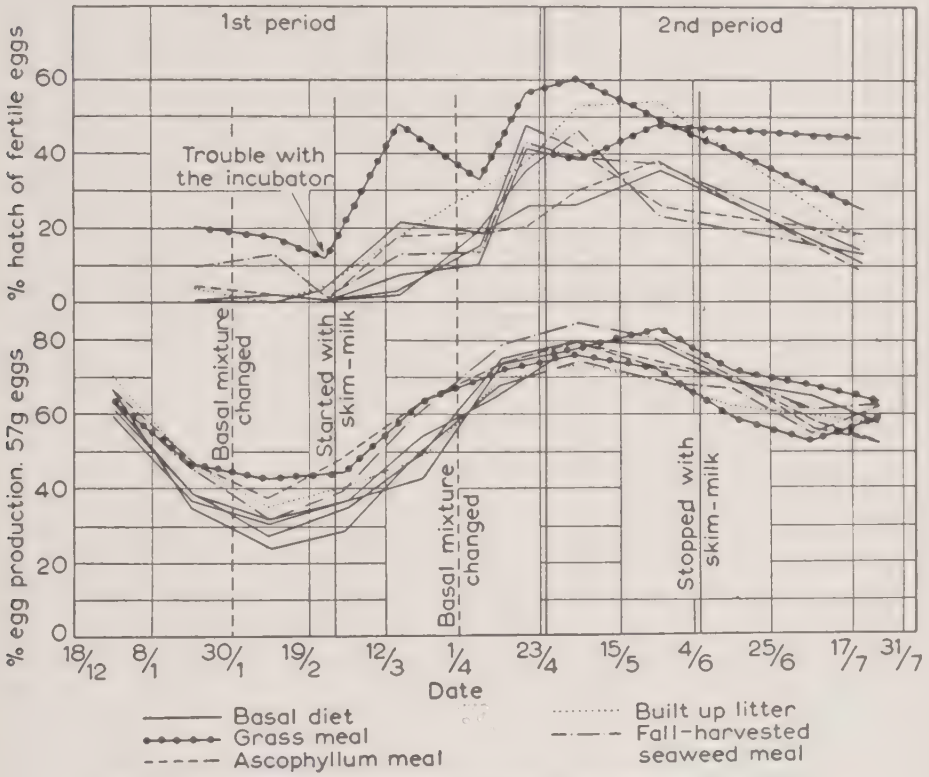


FIG. 6. Egg production and hatchability in experiment No. 232H.

from the built-up litter group for the most part hatched better than the control group and about as well as the eggs from the grass meal group. In the winter months on the other hand there was no special effect from the built-up litter.

The next experiment (402H) lasted 9 months from 9 January to 29 September 1954 and had 10 groups. Four of these had 24 pullets and 6 had 32 pullets.

The concentrate mixtures were as follows:

Groups 1 and 6: Basal diet only.
Groups 2 and 7: Basal diet + 7% grass meal.
Groups 3 and 8: Basal diet + 7% *Laminaria* meal[4a].

Groups 4 and 9: Basal diet + 7% *Ascophyllum* meal[1e].
Groups 5 and 10: Basal diet + 4·5% grass meal + 2·5% *Ascophyllum* meal[1e].

Groups 1–5 received only whole corn and dry mash while groups 6–10 had 50 g skim milk per day as well. All the groups were on built-up litter.

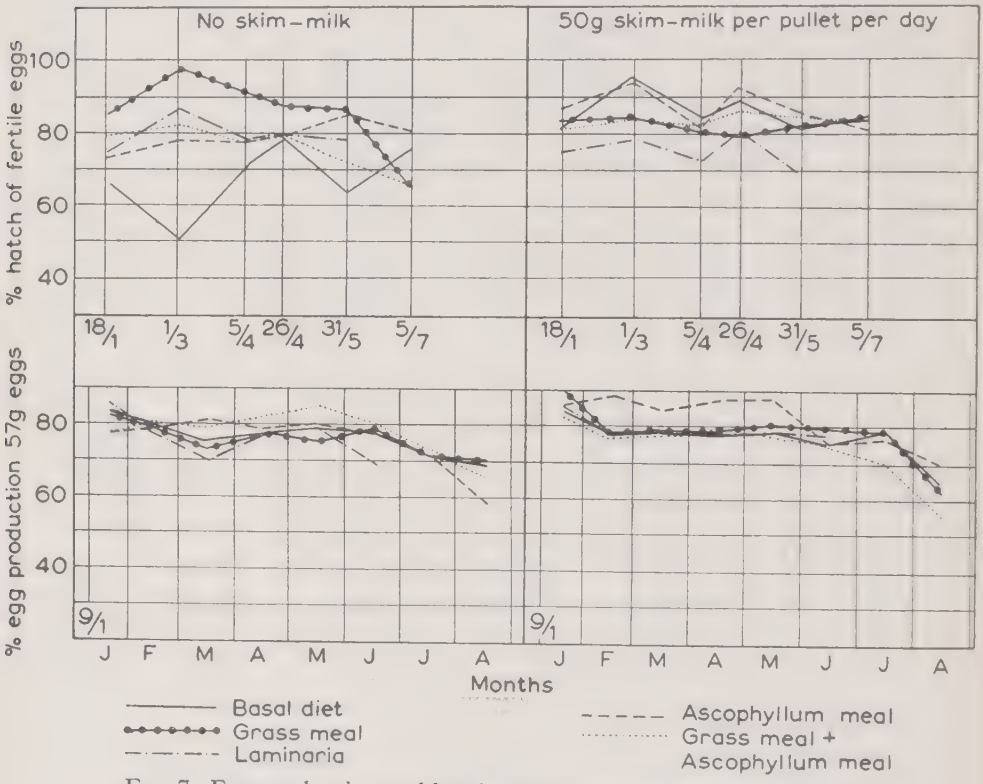


FIG. 7. Egg production and hatchability in experiment No. 402H.

In contrast to the first experiment the laying was good during the whole experiment and no difference found in the groups could be ascribed with certainty to the supplementary feedstuffs.

The eggs from Group 1 (on basal diet) hatched somewhat more poorly than eggs from the other groups (Table 4 and Fig. 7). Supplements of *Ascophyllum* meal[1e], *Laminaria* meal[4a] and special grass meal and skim milk bettered the hatching results. When skim milk was added to grass meal, *Ascophyllum* meal or *Laminaria* meal, no clear difference was found between the different groups (Table 4 and Fig. 7).

Supplements of *Ascophyllum* and *Laminaria* meals gave a slightly stronger yolk colouring than basal diet, but not as strong an effect as grass meal.

The iodine content in the eggs from experiment 402H has been investigated and published by SUNDE[6].

One hundred gram egg yolks from hens in the control groups contained 0·03 mg of iodine. A 7% supplement of *Ascophyllum* meal[1e] to the mash gave an iodine content in 100 g egg yolks of ca. 1·5 mg and 7% *Laminaria* meal[4a] gave 4·5 mg iodine per 100 g of egg yolk.

To test the eggs' storability, samples from different groups were stored for 12–14 months, but there was no indication of any particular effects from the seaweed supplements on either the keeping qualities, odour or taste.

DISCUSSION AND CONCLUSION

In two experiments with chicks (148K and 186K) where well balanced diets were used (Table 2), supplements of *Ascophyllum* meal[1a, 1b, 1c] and *Laminaria* meal[2] gave no significant effect.

In four other experiments (235K, 237K, 239K and 403K) a diet not supplemented with yeast, grass meal or cod liver oil, but which was otherwise balanced was used. Supplements of *Ascophyllum*[1d] and *Laminaria* meals[3, 4a, 4b] gave somewhat the same effect as grass meal in quickening growth and bettering health. The main reason for the bad result in the control groups was believed to be that the basal diet had no supplements rich in riboflavin and therefore contained too little of this vitamin.

The riboflavin content in the basal diet was calculated to be about 1·7 mg per kg. The amount of riboflavin required by chicks 0–8 weeks old is set at 2·9 mg per kg of feed. Hence a clear deficiency of riboflavin in the basal diet itself must be assumed.

Since an addition of cod liver oil emulsion to the basal diet had little or no effect, it is not likely that the rather strong effects of the seaweed meals can be attributed to the Vitamin A and/or D effect of these supplements.

According to these experiments it should be possible in some cases to use seaweed meals in a similar way to grass meal in order to avoid deficiency in certain essential feed components of chick rations.

No positive explanation can be given for the fact that the meal of young lamina of *Laminaria hyperborea* gathered May 9–10 produced a much greater body weight than meal of the same algal type harvested September 20.

Ericson[2] has found that the B_{12} content in *Alaria esculenta* is much higher in the spring than in the autumn, but presently there is little likelihood that the greater effect of spring *Laminaria* meal is due to B_{12}. On the other hand it is natural that the riboflavin content in *Laminaria* should coincide with the B_{12} content, and we think that the strong effect of spring *Laminaria* meal in experiment 403K is related to the fact that the *Laminaria* meal was particularly rich in riboflavin.

It was calculated that the control groups in the first part of experiment 232H on pullets received only ca. 1·57 mg of riboflavin per kg of total feed (Table 2). The norm for laying hens is 2·2 mg/kg of feed and 3·7 mg/kg of feed for hens which are to produce hatchable eggs (National Research Council). The low riboflavin content, together with a somewhat low protein content in the total diet, in all likelihood influenced the poor laying and hatching results.

The supplements of seaweed meals in experiment 232H did not give any clear effect on egg production or hatchability. It is possible that the low protein content in the feed might somehow be connected with the egg laying. The grass meal supplement clearly gave better hatching results.

In experiment 402H the protein content of the basal diet was sufficient from the start, but according to the calculations the riboflavin content was not essentially higher than in experiment 232H, group 4. The improvement of the laying for all the groups, despite this, may have depended on the fact that all the hens were on built-up litter, and that the conditions for the vitamin-producing fermentation in the litter were better than for group 4 in the first experiment, 232H.

At the same time, the basal diet was not rich enough to secure satisfactory hatching, but the results improved when a supplement of grass meal, *Ascophyllum* meal[1e], *Laminaria* meal[4a] or skim milk was administered.

The seaweed meal supplements therefore were able to better hatching results when the hen feed had not been supplemented by yeast or grass meal or when skim milk was given.

Like many other investigations (see SUNDE[6]), the present experiments have shown that the iodine content of eggs can be raised considerably by administering either *Ascophyllum* meal or *Laminaria* meal. In one egg from hens receiving 7% *Laminaria* meal[4a] on their diet, the iodine content measured 18 times man's daily requirement. For eggs from hens which received 7% and 2·5% *Ascophyllum* meal supplements the corresponding figures were 5·4 and 2·5 respectively.

It has been asserted that *Ascophyllum* supplements to hen feed leads to more storable eggs; the present experiments did not support such a theory.

REFERENCES

1. BLACK, W. A. P., *Agriculture*, IX, 126–130 (1953).
2. ERICSON, L. E., *Arkiv för kemi*, **6**, 427 (1953).
3. MACINTYRE, T. M. and JENKINS, M. H., *Sci. Agr.*, **32**, 559–567 (1952).
4. National Research Council, Publication 301, January 1954.
5. ROMIJN, C. and LOKHORST, W. *Tijdschrift voor diergeneeskunde*, **78**, 369–392 (1953).
6. SUNDE, I., Norsk Institutt for tang og tareforskning (Internal report No. 45).

(*For Discussion, see page* 112).

ALGINYL STEARATE

By L. De Keyser (Belgium)

I. OBJECT OF THE PREPARATION

Manufacture of a film-forming product which may be cast from organic solvents to a thin 'coating', resistant to scratches, insoluble in water and soluble in alkaline medium.

II. PREPARATION

Commercial alginic acid is swelled for one hour in diluted HCl. The swelling in water, prior to esterification, has been described before by Cunningham, Chamberlain and Speakman (B.P. 573.591). We have experienced that acidification of this water promotes the esterification. The HCl is washed off with water and the latter is removed by repeated immersions in methanol. The methanol, on its turn, is removed by anhydrous pyridine.

We start from 20 g (13·2% moisture, 0·1 mol) air-dry alginic acid which after pretreatment as described above is immersed into 150 cm^3 anhydrous pyridine. 50 cm^3 (0·4 mol) Stearoyl chloride are thrice added, and the reaction is allowed to continue at 50° C. Time may vary but after 8 hours, 150 cm^3 must still be added since the viscosity of the mass has become too high.

In order to separate the alginyl stearate, the reaction mixture is poured out into HCl 5%. Still more HCl is added until the pyridine odour has disappeared. It is washed with water until it is neutral, and the stearic acid formed is removed by repeated washing, first with cold, then with warm alcohol.

The product obtained is not homogeneous. When it is treated with ether, one part dissolves (product B) and the other part remains undissolved (product A). After separation of the residue A, product B is precipitated in alcohol.

III. ANALYSIS

After saponification of the alginyl stearate by boiling alcoholic KOH for 5 minutes, the potassium alginate precipitates whereas

the potassium stearate remains dissolved. (The potassium alginate is soluble for 0·02%, the potassium stearate for 10% at 66° C.) The separated potassium alginate is further washed with warm alcohol.

After dissolution in water, the product is precipitated with H_2SO_4N. Not all the alginic acid is recuperated since it was partly decomposed during the alkaline saponification. These decomposition products are found again after evaporation of the neutralized filtrate (obtained after filtration of the alginic acid) together with salts. Both can be separated by means of alcohol. The alginic acid is titrimetrically dosed by dissolving it in a determined excess of NaOH, and titrating again with H_2SO_4 N/10 upon phenolphthaleine.

The alcoholic potassium stearate solution is diluted with water to five times its volume, and acidified with H_2SO_4, so that the stearic acid likewise precipitates. It is washed until the rinsing water is neutral. The acid is again dissolved in alcohol, and titrated with NaOH upon phenolphthaleine.

Both products, the soluble and the insoluble, are examined according to this method. To some samples, elementary analysis was applied as well.

In Table 1 the results of the analyses of products, obtained at different reaction times, are given.

Comment to Table 1 :

(1) After 8 hours, the degree of substitution remains unchanged.
(2) There is little difference in chemical composition between the products A and B after 8 hours of reaction. Both of them approach the degree of substitution 2.
(3) The quantity of alginic acid found again is slight indeed on account of the decomposition.

We tried to execute the analysis at room temperature for one night. It appeared, however, to be impossible to separate completely the potassium stearate from the potassium alginate. Even at room temperature, the product decomposes.

IV. PROPERTIES OF ALGINYL STEARATE

When the solution of alginyl stearate obtained after 8 hours of reaction is poured out onto a glass plate, a transparent pale-yellow

Table 1

Duration of reaction	Weight of stearic acid from 1 g ester		Weight of alginic acid + decomposed product from 1 g ester		% C		% H		% O		Average total of the analysis	
	A	B	A	B	A	B	A	B	A	B	A	B
72 h	0·74 0·74	0·76	0·2 0·19 0·16	0·16 0·13 0·11	67·93 67·78 67·83	71·3	10·09 10·13 10·13		17·52 17·59 17·32		95·2	
60 h	0·79 0·78	0·75 0·81 0·79	0·25 0·19	0·18 0·17	68·97 68·86 68·83	68·85 68·62	10·38 10·53 10·65	10·27 10·43	19·86 19·72	20·57 20·34	99·19	99·58
8 h	0·77 0·77	0·76 0·82 0·77	0·2 0·2	0·2 0·23	69·54 69·38 68·55	69·47 69·81	10·39 10·45 10·17	10·57 10·36	17·32 19·31	19·68 19·40	97·84	99·64
6 h	0·74 0·73	0·68 0·67	0·15 0·24	0·15 0·33								

Pure alginic acid calculated	0	1	40·9	4·6	54·6	
Pure stearic acid calculated	1	0	76·06	12·78	11·27	
Degree of substitution 1 calculated	0·64	0·41	65·16	9·5	25·34	
Degree of substitution 2 calculated	0·80	0·25	71·19	10·73	18·08	

plastic film is obtained after evaporation of the solvents. This film has a water-absorption of 0·5%. However, it does not dissolve in alkaline medium. Probably, the stearoyl groups mask the -COOH-groups, so that the latter cannot dissolve, contrary to the alginyl esters of lower fatty acids.

The solubility in organic solvents and the softening point is found in Table 2.

V. OCCURRENCE OF 'CROSS LINKS'

The great difference in solubility between products A and B may be explained by a difference in substitution. Yet, if this difference exists, it is very slight indeed, as appears from the analysis. It is rather explained by cross-linking between the different chains. These cross-links may arise by the action of the -COOH-group of one chain upon the OH group of another. They can likewise arise after the esterification to distearate has occurred already,

Table 2. Solubility and Softening Point

Product	Ethanol		Methanol		Acetone		Dioxane		Ethylacetate		Ethylene chlorohydrine		Tetrahydrofurane		Methylene chloride		Ether		Benzene		Softening point	
	C	W	C	W	C	W	C	W	C	W	C	W	C	W	C	W	C	W	C	W	begin	end
I A	—	—	—	—	—	—	S	S	—	—	—	—	—	—	S	S	—	—	S	S	270° C	273° C
I B	—	—	—	—	—	—	S	+	S	+	—	+	+	+	+	+	+	+	+	+	48° C	54° C
II A	—	—	—	—	—	—	S	S	—	—	—	—	—	—	S	S	—	—	S	S	273° C	276° C
II B	—	—	—	—	—	—	S	+	S	S	—	S	+	+	+	+	+	+	+	+	190° C	210° C
4 A	—	—	—	—	—	—	—	—	—	—	—	—	—	—	S	S	—	—	S	S	270° C	273° C
4 B	—	—	—	—	S	S	S	+	S	S	S	S	+	+	+	+	+	+	S	+	180° C	205° C
5 A	—	—	—	—	—	—	—	—	—	—	—	—	—	—	S	S	—	—	S	S	270° C	273° C
5 B	—	—	—	—	S	S	+	+	—	+	—	+	+	+	+	+	+	+	S	+	145° C	160° C

S = swelling C = cold W = warm

namely by reesterification. As a matter of fact, the products A are quite insoluble in different solvents, whereas the products B dissolve in some solvents (Table 2). Also, according to the duration of the reaction, more A products and less B products are always obtained (Table 3).

Table 3

Reaction time in hours	Product A obtained	Product B obtained	Stearic acid from 1 g A	Stearic acid from 1 g B	C % A	C % B
1	18·85 g	31 g	0·73	0·68	67·3	70·2
2	29·5 g	31 g	0·72	0·71	69	71·7
4	30·2 g	31 g	0·72	0·68	68·2	73·9
6	32 g	18 g	0·75	0·69	70·7	73
8	32 g	13 g	0·74	0·72	72	72·7
48	31 g	8 g	0·77	0·71	69·8	72

For this reason, it is preferable to abridge the time of reaction. From the difference in softening point of A and B, it likewise appears that A is cross-linked by cross-bridges.

DISCUSSION

Dr. Sørensen, Norway, asked Mr. de Keyser if any determinations of the anhydride formation had been undertaken and if he had any information to give about the degree of lactonisation. Mr. de Keyser answered that no such investigations had been carried out.

SEAWEED FOR STOCK FEEDING

By A. A. McInnes (Scotland)

ABSTRACT

Following increased agricultural activity to meet the needs of increasing population in recent years, minerals, particularly trace elements, have been leached out from land areas all over the world, in increasing quantities.

The importance of trace elements is being recognised more widely, while at the same time deficiency diseases attributable to a shortage of trace elements are widespread. In Great Britain it is now difficult to find a farm which is completely free from one or other of the many deficiency diseases the country suffers from.

It is becoming increasingly evident deficiency diseases are more often than not due to a shortage not of one mineral but of several and from this it follows an effect cure must be looked for in a substance containing several minerals.

Seaweed is such a substance. Meals made from blends of several types of seaweed gathered in the North of Scotland are now being used successfully in the rations of cattle, pigs, poultry, and sheep in Great Britain. Farmers report often spectacular improvement in the health of flocks and herds given this meal. Milk fever, mastitis, contagious and spontaneous abortion frequently disappear, calving is easier, calves are healthy and strong, butter-fat content of milk goes up, and examples of experiments are given.

In pigs the quantity and quality of sow's milk improves, recovery after weaning is hastened, piglet anemia often eliminated.

Poultry show benefit through brighter plumage, increased weight, improved general alertness. The percentage of eggs hatching out is often markedly improved.

Started five years ago, progress is sufficient to justify an estimate of the sale of 5000 tons of this meal in the current year.

DISCUSSION

Dr. BERGMANN, U.S.A., made a remark about the deprival of the earth of trace elements and pointed out that a great deal has been done to remedy the loss. As examples he mentioned the addition of cobalt to Australian soils and of boron to the soils of Florida.

(*For further discussion, see page* 113).

PRODUCTION OF MANNITOL AND LAMINARIN FROM SEAWEED AQUEOUS EXTRACTS

By Kenneth C. Reid (Scotland)

ABSTRACT

From an economic viewpoint, only *Laminaria cloustoni* frond, cut in the months July to October from the coasts of Britain, is suitable for commercial production of mannitol and only that cut in August to December is suitable for production of laminarin. To produce both these compounds and to conserve equipment and labour the processes should coincide as far as possible. By using a suitable aqueous extraction technique, extracts suitable for production of either or both compounds may be obtained. Fractional crystallisation of mannitol from the aqueous extract is made possible by removal of interfering laminarin and fucoidin by precipitation with methanol. Laminarin may then be recovered from the precipitate.

DISCUSSION

Dr. Gardner, Scotland, asked Mr. Reid if the starting material was dried or fresh and if the speaker would suggest a separation into fronds and stipes outside the period when the fronds are rich in laminarin and mannitol, the fronds being used as fodder for cattle and the stipes processed separately. To the first question Mr. Reid answered that he had used dried fronds in order to get a rich solution without concentration of the extract; the economy of the process depended on this. The calculations were based on the assumption that the harvesting of stipes for alginate production was itself economic and consequently the fronds would be available for other use without additional harvesting cost. To a question by Dr. Gardner about the proportions of the components in stipe and frond Mr. Reid said that apart from the difference in the amount of alginate between stipe and frond it would presumably be better to harvest as much stipe as needed for the alginate production than to produce an equal amount of alginate from whole plants.

Dr. Lindberg, Sweden, wanted to know if Mr. Reid had tried weak hydrolysis in order to get rid of some of the glycosides and acetates present in the solution. This would certainly facilitate the crystallisation of the mannitol. Mr. Reid replied that this good idea had not been tried.

Mr. FLOOD pointed out that the maximum content of laminarin occurred at a time of the year when open air drying of the seaweed seemed to be very difficult to carry out and he wanted to know if Mr. REID had taken this into consideration. Mr. REID answered that weed for laminarin and mannitol production had to be artificially dried anyway. He did not think that anybody had succeeded in air drying of weed for this purpose. To a question from Mr. FLOOD on whether he had taken the cost of drying into account, Mr. REID replied that he had. Mr. FLOOD mentioned that the artificial drying of Laminaria fronds was quite a difficult problem, but Mr. REID thought that this problem had recently been solved quite satisfactorily by pulping and film-drying.

Dr. ANDERSEN, Germany, inquired about the length of the ageing period before crystallisation took place. Mr. REID stated that the laminarin would not precipitate from a water solution without any acid present until 3 to 4 days. This precipitate would not dissolve in cold water when dried with alcohol-ether, contrary to a precipitate dried by heating.

THE USE OF *Macrocystis Pyrifera* AS SOURCE OF TRACE ELEMENTS IN HUMAN NUTRITION

By G. L. Seifert and H. C. Wood (U.S.A.)

Read by G. L. Seifert

ABSTRACT

A study of 400 obstetrical patients was made in which 5·5 gram tablets of *Macrocystis pyrifera*, fortified with cobalt and folic acid, were the only source of trace elements used.

It was found that the majority of these young patients were suffering from well established secondary anemia, when they first presented themselves for care. Within six to eight weeks on three tablets per day the haemoglobin levels had reached an average of 12 mg (85%). Furthermore, in patients who stopped this food supplement during a period of time, there was a rapid drop in haemoglobin levels which rose again with resumption of the tablets.

In all patients studied there was a spectacular drop in the incidence of colds. In those colds which were contracted, the intensity, and the duration, were so much reduced that the annoyance of the infection was minimal. The factor or factors responsible for this effect are not clear but it is supposed that the general improvement in the body metabolism is responsible.

In all patient groups, and particularly the geriatric group, there was a noticeable improvement in physical stamina ('4 o'clock fatigue' being ameliorated), this probably being due to the beneficial effect of the iodine content on the functioning of the thyroid gland.

In the practices of both authors the rate of miscarriage is well below that of the population at large. In patients who came with a history of miscarriage the use of this supplement has apparently been successful. The combination of manganese and cobalt in the tablets are thought to be major factors in this observation.

The functioning of the G.I. tract was very much improved,

digestion, elimination, lack of scouring or constipation from the iron content, etc.

There is in the various organs of the body unusual concentration of essential elements and the authors are convinced that future research will show the same relation to the glands involved as that of iodine to the thyroid.

DISCUSSION

Dr. Aleem, Egypt, asked if the people taking part in the experiment were specially chosen from different classes of the community. Dr. Seifert replied that the experiment was based on an average American population from an industrial district.

Mr. Richardson, Scotland, inquired about control groups given dummy tablets. Dr. Seifert denied that they had used any controls this time. Earlier tests had given effects corresponding to a 5 to 6% earlier rise than the controls. Dr. Young wanted to know what amounts of trace minerals might have been given during these experiments. Dr. Seifert stated that no arsenic was present as the formula of these tablets had been accepted by the Food and Drug Administration. Analyses for fluorine had also given negative results.

Mrs. Kylin, Sweden, asked if any vitamins were added to the diet, to which Dr. Seifert replied that no other growth substances were given.

Dr. Aleem inquired about the psychological effect in such an experiment and if it had been thoroughly eliminated. Dr. Seifert said that this part of the problem had not been taken into account. He then pointed out the importance of the trace minerals and indicated that some of the effects caused by vitamins might possibly be due to the trace elements present. This ought to be further investigated.

THE IODINE CONTENT OF EGGS FROM HENS FED WITH SEAWEED MEAL

By I. Sunde (Norway)

Read by B. Larsen (Norway)

It is reported in the literature that eggs from hens fed on a diet rich in iodine show a considerable increase in iodine content. Several experiments on the production of such eggs were carried out in the early 1930's in connection with studies on curing of goitre by means of iodine. The results of some of these investigations are shown in Table 1. The first column shows the iodine content of eggs from hens fed on a usual diet, for the sake of convenience called ordinary eggs, given as μg per egg. The agreement

Table 1

	I_2 in ordinary eggs, μg per egg	Daily I_2 dose, mg per hen	Iodine source	Iodine content per egg	
				μg	mg per mg daily iodine dose
1. Viollier and Iselin	1·3–3·4	0·17	iodized cod-liver oil	34–82	0·2–0·48
2. Jaschik and Kieselbach	1·2	1·5	KI	150	0·1
3. Scharrer and Schropp	4–7	2	I_2 dried kelp	300–400	0·15–0·2
4. Wilder *et al.*	—	2–5	iodized linseed meal, KI	—	0·15–0·2
5. Simpson and Strand	9	8	KI	375	0·05
6. Berkesy and Gönczy	5–18	—	KI	400	—
7. Leone	—	24–432	NaI and KI	1680	0·07–0·004
8. Peano and Pissaro	9·7	40	Organic I_2-com.	2165	0·05
9. Straub	4·5–6·8	—	—	2500	—

between these determinations is not good. The next two columns show the daily iodine dose in mg per hen and the source of iodine used by the different authors. In most cases inorganic iodine has been used. Compounds containing organic bound iodine have been fed by Wilder *et al.* as iodized linseed meal, by Viollier and Iselin as iodized cod liver oil, and by Peano and Pissaro. In only one case has the iodine source been kelp meal. The next column shows the iodine content of the eggs produced in μg per egg. When

compared with the figures in column 1, the increase in the iodine content is obvious. In the last column, we have calculated the iodine content per egg per mg daily iodine dose. This gives an approximate measure of the fraction of the daily iodine dose which is stored in the egg.

From the table it is seen that the figures of columns 2 and 4 increase on reading from top to bottom while those of the last column seem to increase. This means that an increase in the daily iodine dose leads to an increase in the iodine content of the eggs but to a decrease in the iodine content per mg daily iodine dose. In other words: The amount of iodine in the eggs will increase when the daily iodine dose increases, but a smaller fraction of the iodine fed will go into the eggs. This conclusion seems to be rather probable and also to be supported by the results to be reported here.

As mentioned before, the iodine content of ordinary eggs varied from 1·2 to 18 μg per egg in the experiments shown in Table 1. This variation is probably due to the inconvenient analytical method used by these authors. They have used the dry combustion technique and colorimetric or titrimetric determination of iodine in the ash. We have also tried this method, but found it very laborious and inconvenient.

To estimate the iodine content of the eggs we have used the method proposed by CHANEY for determination of iodine in blood serum as modified by KIRKPATRICK. In this method, organic matter is destroyed and iodine, in all forms, is oxidized to iodate by digestion with a mixture of chromic and sulphuric acids. The iodine is volatilised by reducing the digest at boiling point with phosphorous acid and trapped in an absorption solution in a special still. The amount of iodine in the absorption solution is then estimated by means of its catalytic effect on the reduction of ceric sulphate by arsenious acid in sulphuric acid solution. The reaction takes place in the following way: $2\,Ce^{\cdots\cdot} + As^{\cdots} \rightarrow 2\,Ce^{\cdots} + As^{\cdots\cdot\cdot}$. The rate of reduction is proportional to the amount of iodide present within a narrow range in the iodide concentration. The amount of iodide can thus be estimated by measuring the optical density of the colour due to the remaining, unreduced ceric ions and comparing with a standard graph.

In the present experiment, seaweed meal was fed to 6 groups of hens in addition to the basal ration as just reported by Professor

HØIE (p. 82 this volume). Two groups got 7% seaweed meal of *Laminaria hyperborea* (0·41% I), another two 7% meal from *Ascophyllum nodosum* (0·07% I) and the last two 2·5% of the same *Ascophyllum* meal. The eggs were collected for analyses when the hens had been fed on this diet for 4 to 5 months.

Several authors have reported that iodine is stored mainly in the yolk. Preliminary analyses of some eggs from this experiment confirmed these observations. Two eggs analysed contained 1500 and 1000 μg iodine per 100 g yolk and only 140 and 90 μg respectively per 100 g white. Therefore, we have only estimated the iodine content of the yolk and calculated the content of the whole egg from the relation mentioned above. The results are shown in Table 2.

Table 2

Daily I_2-dose, mg per hen	Iodine source	Iodine content per egg	
		μg	mg per mg daily iodine dose
0	—	6	—
1·14	2·5% *Asc. nod.*	125	0·11
1·27	2·5% ,, ,,	180	0·14
3·45	7% ,, ,,	230	0·07
3·4	7% ,, ,,	270	0·08
20·6	7% *Lam. hyp.*	870	0·04
20·6	7% ,, ,,	910	0·04

The figure 6 in the first line of column 3 is the iodine content of eggs from hens fed on a usual diet. This is in good agreement with the results which were shown in Table 1. With a daily iodine dose ranging from 1·14 to 20·6 mg per hen, the iodine content of the eggs ranged from 125 to 910 μg per egg. A good agreement is observed between groups getting the same diet. As in Table 1 it is seen that an increase in the daily iodine dose leads to an increase in the iodine content of the eggs (columns 1 and 3) but to a decrease in the fraction of the daily dose stored in the egg. This seems to be a safe conclusion to draw from this experiment.

Many of the authors mentioned in Table 1 have reported that the iodine present in the yolk is partly organic and partly inorganic bound[2, 6, 9]. STRAUB claims that 25% of the iodine is bound to the

proteins while JASCHIK and KIESELBACH claim that it is bound in the lipoid fraction. The methods used for these determinations are not reported. We therefore found it necessary to carry out some investigations on the iodine content of different fractions of the yolk.

The proteins were precipitated from a suspension of the yolk in water by means of ethanol or zinc sulphate and alkali. No iodine could be detected in the precipitate. Analysis of the supernatant liquid showed an almost complete recovery of the iodine. Parallel determinations on different eggs gave the same result.

The supernatant liquid was then shaken with a suspension of silver carbonate in water and centrifuged. Analysis of the liquid fraction showed that all iodine was removed from the water phase by this treatment.

The lipoid fraction was extracted from the yolk by means of chloroform. No iodine could be found in the extract. Use of ethyl ether instead of chloroform gave a fairly large amount of iodine in the extract, but this was found to be due to peroxides present in the ether.

We have thus been unable to find any iodine in the protein or lipoid fraction. All iodine is water soluble and will be precipitated by silver carbonate. No difference was found between eggs from hens given equivalent amounts of iodine as seaweed meal or potassium iodide. This does not necessarily mean that iodine is inorganic bound in the eggs. Insufficient methods will delay the solving of this problem.

DISCUSSION[1]

Dr. ROMIJN, Holland, gave a summary of the distribution of iodine in eggs from laying hens. Feeding experiments with seaweed meal resulted in a rapid increase of the iodine content of the eggs after a period of 4 days after the addition of the meal to the ration. This rapid increase continued until approximately the 12th day when the maximum content was reached, and this level was maintained as long as the seaweed meal was given. This was in full accordance with the physiological mechanism of egg formation in hens as it takes about 10 days to complete the yolk. The eggs laid during the first 4 days already had a completed yolk before the seaweed meal was added, and therefore no increase in iodine occurred. From the 4th to the 10th day an ever increasing part of the yolk was formed during the seaweed feeding. This explained why the iodine content increased during this period. After the 12th day the whole egg was

[1] Refers also to the papers by HØIE and Sandvik, p. 82 and MCINNES, p. 103.

formed during the seaweed period and the maximum iodine content was reached and then maintained. It did not make any difference whether seaweed meal or iodine in the form of potassium iodide was given. This might indicate that the resorption of iodine, and possibly the other trace minerals from the seaweed meal, is not limited by the digestibility in the intestinal tract of the fowl. During the first four days the iodine found in the eggs was mainly concentrated in the shell. In the stable period 60–70% was found in the yolk and approximately 30% in the shell. No increase in the iodine was observed in the white or the shell membrane.

Dr. SØRENSEN, Norway, asked Mr. MCINNES what biological experiments had been carried out to establish the preference of the mixed meals over the single ones. The experiments of Dr. ERICSON and Dr. LUNDIN made it tempting to try mixed meals, but he was afraid that some of the species might be less favorable especially as there are some which are said to be toxic like *Laminaria Gunnerii*. Mr. MCINNES stated that he knew of no official scientific control experiments. His conclusions were based on his own practical feeding experiments with mixed and single meals to domestic animals.

Dr. WOODWARD, Scotland, congratulated Dr. HØIE on his excellent experiments and inquired what type of *Laminaria* meal was used. Mr. HAUG, on behalf of Dr. HØIE, stated that the meal was made from *Laminaria hyperborea* fronds, and he suggested that the difference between autumn and spring samples might be caused by the higher content of B vitamins, especially B_{12}, of the latter.

Dr. GARDNER, Scotland, pointed out that the difference in the duration of the experiments had to be taken into account when judging the results.

Mr. FLOOD, Ireland, questioned the possibility of the mineral matter being chemically combined with organic compounds. He pointed out that the evidence for the existence of such compounds was not quite convincing with the possible exception especially in the case of iodine and that the mineral substances appeared to be present as inorganic compounds. Mr. MCINNES told that practical feeding experiments carried out by his friends and himself had proven the superiority of seaweed meal over artificial mixtures of minerals and vitamins. He admitted that scientific experiments with control groups were not carried out.

Mr. TORGERSEN, jr., Norway, wanted to know if there might be any relation between the colour and blood-stains of the egg yolk and the iodine content. Dr. ROMIJN replied that hens given potassium iodide or other iodine salts did not develop any red colour in the yolk.

A COMPARISON OF THE PROPERTIES OF VARIOUS PREPARATIONS OF SODIUM ALGINATE

By D. L. Vincent, D. A. I. Goring and E. Gordon Young (Canada)

Read by E. Gordon Young

ABSTRACT

Fifteen specimens of sodium alginate from various brown algae, including species of *Laminaria*, *Fucus* and *Ascophyllum*, have been examined for optical rotation, ionic mobility, rate of sedimentation, viscosity, and content of ash. Samples varied from 61·7 to 99·6% in content of alginate. Rate of sedimentation was strongly dependent upon concentration. Mobility varied only slightly. Viscosity varied widely and was the best criterion of state of polymerisation. Physical constants were $[\alpha]_D^{20}$ – 133° to 140°, μ 2·9 to 3·1 $\times 10^{-4}$ cm² at *p*H 5·5, I 0·05 and 25°, s_{40} 1·3 to 1·6 S (c=0·5%), η 2 to 20 *cs* at 25° in 0·1N NaCl (c=0·25%), and $[\eta]$ 3·6 to 19·2. Particle weight varied from 42,000 to 222,000. The specific refractive increment was 0·160 g^{-1} cm^3.

DISCUSSION

Dr. McDowell, England, wanted some more information on the lower limit of the molecular weight. He pointed out that the thermal depolymerisation proceeded readily down to approximately 20,000, but was found to be very slow below that point. Dr. Young said that as they were interested in the high viscosity preparations they had not been working with the low molecular forms or thermal depolymerisation.

Mr. Haug, Norway, was interested in the viscosity measurements. He pointed out the difficulties in obtaining a Newtonian flow and asked Dr. Young how these problems were solved by him. Dr. Young stated that they had been very cautious about the viscosity measurements and the intrinsic viscosity was obtained from a series of viscosity determinations by extrapolation to zero shear stress, but they were not quite convinced that they could extrapolate to zero with a reasonable degree of accuracy.

Mr. Kristensen, Norway, had noticed that the properties of the alginate from different species shown by Dr. Young usually did not vary to

any large extent, but in the case of *Laminaria cloustoni* an optical rotation of 140° was considerably higher than the 130° observed for *Laminaria digitata*. He asked Dr. YOUNG if he considered this to be characteristic of the species involved. Dr. YOUNG replied that they had been looking for relationship between species and physical properties but he did not think they had established any particular relationship.

Dr. ANDERSEN, Germany, wanted to know if Dr. GORDON YOUNG from his observations could say anything about the degree of branching of the molecule, to which Dr. YOUNG replied that the physical measurements they had undertaken did not allow any such conclusions to be drawn. Dr. SØRENSEN, Norway, was astonished by the high viscosity reported for the alginic acid isolated from *Fucus vesiculosus*, almost as high as that from *Laminaria digitata*. Contrary to this, at the Norwegian Institute of Seaweed Research one had always found a much lower viscosity of the extracts from the rockweeds than from *Laminaria digitata*. In addition, the alginic acid from the rockweeds had proven to be quite unstable so that a loss of viscosity occurred already during the preparation. He wanted to know how Dr. YOUNG had managed to obtain such a high viscosity alginate from *Fucus vesiculosus*. Dr. YOUNG presented two preparations of alginic acid to the audience: one snow white sample which required a somewhat extended method of preparation and a crude, yellow one prepared commercially by a very simple method. The latter was contaminated with considerable amounts of fucoidin. This might be the cause of the high viscosity of this sample.

Section III

METHODS FOR ANALYSES AND EXPLOITATION

ON THE DETECTION OF ALGINIC ACID IN TISSUES BY MEANS OF BIREFRINGENCE

By Geert Andersen (Germany)

Our research work on alginic acid (aa.) was concerned with the birefringence properties of this polyelectrolyte. We have found that the positive sign (or character) of its intrinsic double refraction is intensified by a positive textural double refraction or 'Form-doppelbrechung'. This is remarkably contrary to the structurally related pectinic acid, whose negative intrinsic double refraction is superposed by a textural birefringence even when only slightly swollen. For the understanding of what follows it is necessary to go back a little[1, 2, 3].

A gel is birefringent when its birefringent micelles are ordered. This is a consequence of optical summation. Such an orientation of particles takes place when an ionotropic gel is formed, according to Thiele 1947[4]. If a drop of electrolyte with bivalent counterions, for instance $CaCl_2$, is added to a one per cent solution of sodium-alginate in a shallow dish with isotropic glass bottom and is left alone for some time without any convection, a circular gel-disc is slowly formed by the centrifugal ions stream. The reason for this chemogen gelformation is the heteropolar, primary valence cross linkage of the more or less stretched molecules by ion-exchange. Thus the alginate is partially discharged and dehydrated. The micelles are arranged perpendicular to the direction of the ionic flow and fixed there. Fig. 1 shows spheric discs of copper-alginate with concentric arrangement of particles. The crossed axis of optically monoaxial crystals can be seen and the sectors are coloured with gypsum red first order. According to the terminology of Frey-Wyssling[5] the character of these spheric discs is negative with reference to the diameter.

The formation of ionotropic gels is an ordered multiple membrane formation. At the zone of contact between solution and electrolyte a membrane is immediately formed. Through this more

ions diffuse, forming further membranes as soon as their concentration reaches the limiting coagulation value. Statistically, the particles in the ionotropic membrane are arranged irregularly in the square dimension. The membrane therefore appears, in this direction, isotropic; in cross-sections of any direction, however, with a positive sign of birefringence with reference to the length. The membranes themselves link up slightly and thus form a gel which also has mechanical anisotropy. The sign of birefringence of aa. is, because of the identity of both kinds of double refraction, a material constant. If it is known, conclusions can be drawn on respective arrangement of particles. The degree of order in ionotropic gels can also be proved by means of electron microscope or X-ray analysis.[9]

These ionotropic alginate gels are organized micellated structures. Because of their double refraction, swelling-anisotropy and ion-exchange-capacity, they may be compared with biological gel structures. We have investigated the most important regularities for the formation of ionotropic gels, i.e. the dependency on the chain-length, the grade of dissociation and the contents of accompanying substances.

Ionotropic gels are heteropolar primary valence gels. The ordering effect of counterions on the aa. particles is specific to the respective ions. An ionotropic lead-alginate gel is more birefringent than a calcium-alginate gel, and ion-series can be formed. Lead-ion dehydrates alginate more effectively than Ca-ion; this leads to higher birefringent gels. Swelling and birefringence are inversely proportional. The rate of birefringence is directly connected with the discharging or saturation rate of carboxyl groups, which takes place spontaneously during gel formation. This occurs only if no higher degree of saturation has been forced by high electrolyte excess. At equal conditions lead discharges more COOH-groups than calcium. A high rate of birefringence of ionotropic gels therefore means great affinity of counter-ions, high degree of saturation of COOH-groups, low swelling and strong gel stability.

Ionotropic alginate gels are shrinking and swelling anisotropic. They react by ion-exchange with variation of birefringence. If, therefore, the hypothetic linkage COO—Cu—OOC is replaced by a hydrogen bond, an anisotropic shrinkage of the structure takes

FIG. 1. Ionotropic Cu-alginate-gel spheric-discs in polarized light. The centrifugal ions-stream causes perpendicular arrangement of alginate particles and birefringence.

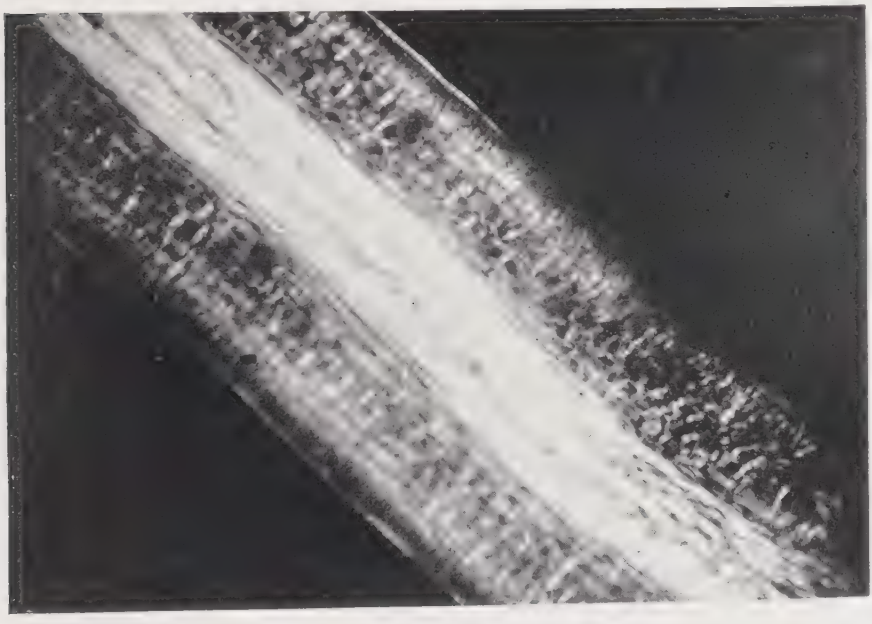


FIG. 2. Cross-section of the Lamina of *Laminaria sp.* For explanation see text. 36 ×.

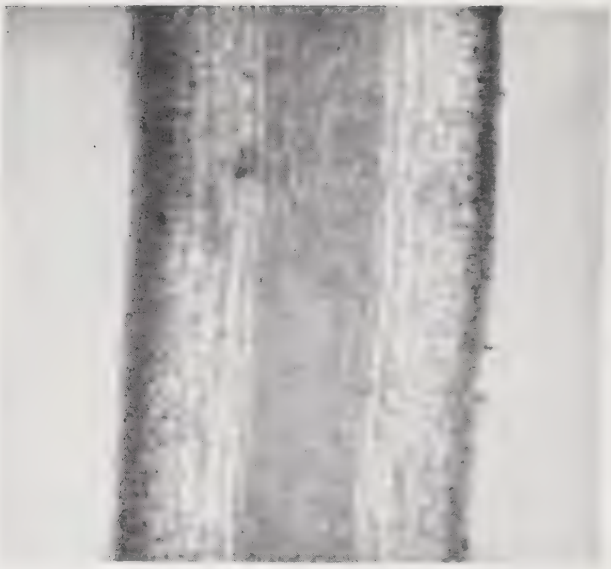


FIG. 3. Cross-section of the Lamina of *Laminaria sp.* in polarized light with gypsum red first order. 36 × .

FIG. 4. Cross-section of the stipe of *Laminaria sp.* in polarized light. Swelling in sodium-citrate solution.

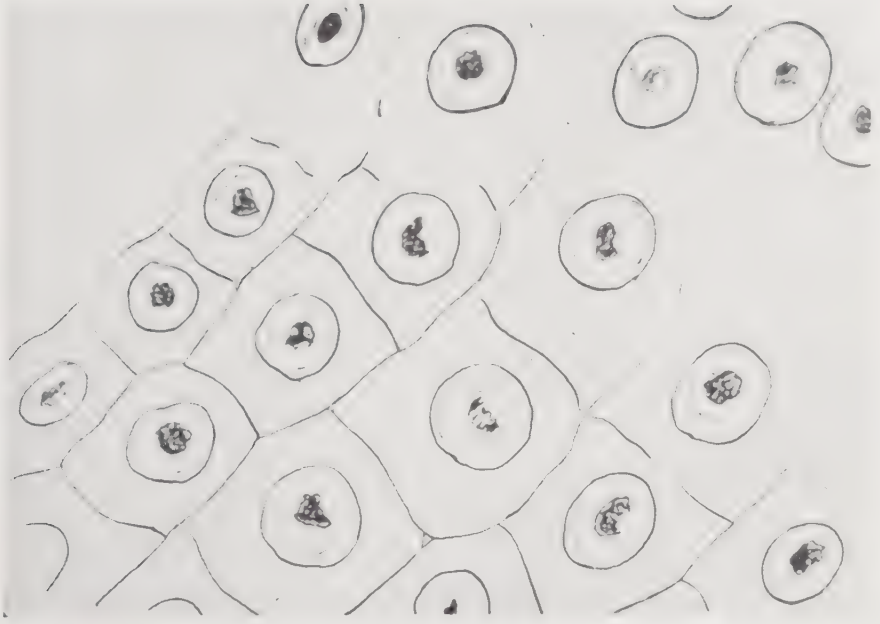


FIG. 5. Diagram of the zone of swelling in a cross-section of the stipe of *Laminaria sp.* For explanation see text.

place, and the degree of order, i.e. double refraction, increases. Ionotropy, however, is not only a dehydration, for substances like glycerol, alcohols or urea do not cause or increase any double refraction.

Experiments with brown algae which were made in co-operation with Prof. C. HOFFMANN KIEL[6], showed that the regularities for the formation and the behaviour of ionotropic gels are also valid for organized biological alginate gel-structures.

As far as we know, polarization microscopic investigations on brown seaweeds had not previously been carried out. Probably the described isotropy of pectinic acid in the protopectin caused it to be assumed that aa. would also be isotropic. Furthermore, the birefringence of brown algae sections has probably been ascribed to cellulose alone because there was no way of differentiation, since cellulose and aa. have the same sign of birefringence. Under conditions which leave the birefringence of cellulose unchanged, the birefringence of the aa. can be varied by means of ionotropic reactions or anisotropic shrinkage. Upon this is based our method of polarization optical detection of aa. in tissues. In this way we tried to determine how aa. is distributed in tissues, and whether or not it is a membrane constituent. Even the untreated section of the lamina of *Laminaria* gives an impressing picture (Fig. 2). On the outside there is some sort of cuticula of waxlike character. The vicinated cell-layers as a whole up to the medulla appears negatively birefringent with reference to the length, because of the tubular structure of these cells. The cell walls of the elongated cells increase in thickness towards the centre. Therefore striating cords become positively birefringent (Fig 3). The medulla is completely positive. The cellulose has, of course, the major share of the whole birefringence. Added mineral acid, however, effects an increase in double refraction as a whole, and in the birefringent areas and in the clearness of the contours and structures. For the increase in double refraction the following data have been found:

Cortex	80–91%
Medulla	up to 150%

The measured double refraction of non-acid treated sections is usually smaller in the medulla than that of the cortex, and is dependent on the thickness of the object. The increase of the double

refraction, when acid is added, is, however, not dependent on this. This increase in the medulla is greater than in the other parts of the tissue. This is to be ascribed solely to the presence of polyuronic acids, because the considerable birefringence of cellulose influences only the absolute level, but not the relative fluctuations due to pH-variations. Therefore we can furthermore conclude that the percentage proportion cellulose to alginic acid in the medulla predominates strongly in favour of aa. The tissue becomes richer in alginate towards the centre. This increase seems to take place at the same rate at which the thin-walled epidermal cells are rebuilt, via thickwalled cortex cells, to highly swollen medulla elements.

Moreover, we know from our experiments that an increase in birefringence with acids takes place only when alginate gels are those of polyvalent cations. The brown algae therefore cannot consist mainly of aa. or salts of monovalent cations. From the data for the relative increase in birefringence after acidification, we can conclude that the aa. in brown algae is present mainly as incompletely saturated salts of alkaline earth metals. We are thus able to confirm the results of WASSERMANN[7], obtained by means of ion exchange.

In order to find out whether aa. is a cell wall constituent, we investigated the extraction process by means of a polarization microscope. The separate stages of the extraction process can best be observed and fixed if acidified and well washed sections are left to swell in a very weak sodium-citrate solution (Fig. 4). The illustration shows a cross-section of *Laminaria* by polarized light. The cells on the right hand side are already separated from the formation. They are surrounded by the secondary membrane and show a distinct spheroid character. The continuity of the primary membranes makes them look like birefringent fibrils, which run from the right to the left. In the zone of swelling, which moves slowly towards the epidermis, they are dissolved. Greater enlargements of the zone of swelling are difficult to reproduce, since, due to swelling, the contours are not clear. We have therefore made a special drawing of the swelling zone (Fig. 5). From this it can be seen that the middle lamellae and the primary membranes, using the terminology of FREY-WYSSLING (1940)[8], are completely swollen and that the cells, now only surrounded by the secondary membrane, are separating from the cell formation. Their shape

now corresponds exactly to the borderline between primary and secondary membrane, which can be seen on the left hand side.

These cells give pure staining reaction for cellulose. The following observations yield further proof that they do consist of cellulose:

1. The separated cells do not swell with weak alkalines. Alginate gels do so.
2. The birefringence of the secondary membranes is higher than that of the primary membranes and is influenced neither by acids nor by alkalines.
3. The light refraction of the secondary membrane during all stages of swelling is stronger than that of its surroundings. This can be derived from the migration of the Becke-line.

These findings are confirmed by a staining reaction. With iodine and 72% sulphuric acid, cellulose becomes a deep blue colour, and the aa. becomes yellowish-brown and shows a distinct dichroism to colourless. These sections have to be observed quickly, for within half an hour the strong acid has completely hydrolyzed or oxidized the aa. In polarized light, however, while all blue tissue is birefringent, *not all birefringent tissue is stained blue.*

SUMMARY

Our investigations show that aa. in tissues varies its birefringence by ion-exchange reactions as in our model-experiments. A polarization optical differentiation between cellulose and alginic acid was therefore possible. By these results, and by staining reactions, we have been able to localise alginic acid in tissues. In analogy to the pectic substances in higher plants, the alginic acid is situated in the middle lamella and the primary membrane, i.e. in the so-called composed middle lamella. In contrast to pectinic acid, alginic acid is not associated with considerable amounts of insoluble substances.

REFERENCES

1. Thiele, H. and Geert Andersen, *Kolloid-Z.*, **140**, 76 (1955).
2. Thiele, H. and Geert Andersen, *Kolloid-Z.*, **142**, 5 (1955).
3. Thiele, H. and Geert Andersen, *Kolloid-Z.*, **143**, 21 (1955).
4. Thiele, H.: *Naturwiss.*, **34**, 123 (1947).
5. Frey-Wyssling, A., *The Submicroscopic Morphology of Protoplasm, etc.*, New York-Amsterdam, 1948.
6. Hoffmann C. and G. Andersen, *kieler Meeresforschungen*, IX, **149**, 1955.
7. Wassermann, A., *Ann. Bot.*, **12**, 137 (1948).
8. Frey-Wyssling, A., *Naturwiss.*, **28**, 385 (1940).
9. Thiele H. and Geert Andersen, *Z.f. Naturforsh*, **106**, 677 (1955).

DISCUSSION

Dr. CHESTERS congratulated the speaker on the most interesting results. Dr. YOUNG reported that experiments of somewhat the same sort had been carried out in his laboratory for the localisation of the polysaccharide in *Chondrus* and the carrageenin was quite definitely shown to be an intercellular substance. Mr. FLOOD commented on the remarkable photomicrographs shown by Dr. ANDERSEN in demonstrating the distribution of cellulose and alginic acid in the plant structure. He had himself demonstrated alginic acid in the cell wall of *Laminaria* by reacting to form the copper salt and then staining with hydrogen sulphide, but owing to the black stain had been unable to distinguish the cellulose constituent.

ON THE QUANTITATIVE DETERMINATION OF ALGINIC ACID

By A. Jensen and I. Sunde (Norway)

Read by A. Jensen

As alginic acid is the only constituent which at the present time is processed on any industrial scale from brown algae, the quantitative determination of this compound becomes very important. We have to admit that up to this day no really good method has been available which is at the same time reliable and convenient for routine analysis.

Below are listed the methods which have been or may be used for determination of alginic acid:

1. Gravimetric Methods, based on the free acid or its insoluble salts.
2. Titrimetric Methods, making use of the acidic property of the compound.
3. Colour Reactions of the furfural formed by treatment with strong mineral acids.
4. Determination of the carbon dioxide evolved by treatment with strong mineral acids or by thermal decarboxylation.
5. Methods based on the consumption of a more or less specific oxidising agent.
6. Polarimetric Determinations, making use of the optical activity of the compound or its derivatives.
7. Methods based on colloidal properties of the acid or its derivatives, as metachromatic effect, by colloid titration or by turbidity measurements.
8. Bioassays based on specific organisms or enzymes.

We shall limit ourselves to the three more important methods, namely the titrimetric, the colorimetric, and the decarboxylation method.

The titrimetric method has been used extensively for the determination of acid groups of fibrous materials. Lüdtke introduced the calcium acetate modification which was investigated by

McGEE, FOWLER, and KENYON (*J. Amer. Chem. Soc.*, **69,** 347 (1947)). The method was found to give somewhat lower values than the decarboxylation value and the theoretical amount (23·1—25·3—25·56% COOH respectively).

CAMERON, ROSS and PERCIVAL (*J. Soc. Chem. Ind.* **67,** 161 (1948)) worked out a detailed procedure for the routine determination of alginic acid based on the calcium acetate method. No experiment was carried out to establish the relationship of their method to the decarboxylation procedure or the theoretically expected values. Only an agreement with the direct titration method was confirmed. In this laboratory we have checked the direct titration method, using a somewhat modified Cameron-Ross-Percival procedure. The method was found to be rather time-consuming, requiring a very careful treatment of every single analysis, but then giving results reproducible to within one per cent of the mean value.

The colorimetric reaction of carbazol with furfural formed from uronic acids by decarboxylation has long been used for the determination of these acids. PERCIVAL and ROSS (*J. Soc. Chem. Ind.* **67,** 420 (1948)) developed a procedure for the determination of alginic acid based on this reaction. We followed this procedure with a few alterations during the present study.

In order to make the method reproducible, a very rigorous standardization of every step of the procedure had to be introduced, and the reaction itself is not a quantitative one, giving about 34% of the theoretical amount of furfural.

The titrimetric and the colorimetric analyses both involve an extraction of the alginic acid of the sample, and it is of course essential that this extraction be quantitative.

The decarboxylation of uronic acids with strong mineral acids has been investigated by a large number of workers, leading to many modifications of LEFEVRE's and TOLLEN's original method (*Ber.* **40**, 4513 (1907)). We followed a procedure worked out by MCCREADY, and collaborators (*Ind. Eng. Chem., Anal. Ed.*, **18,** 290 (1946)). Carbon dioxide from carbonates was first driven off, and the decarboxylation was then carried out by means of 19% HCl at 110° C. The carbon dioxide was determined gravimetrically. Differences between duplicate runs were found to be insignificant, and the carbon dioxide production was quantitative.

As the colorimetric method has to be standardized against another determination, it is of course of interest to compare only the two more absolute methods. Table 1 gives a series of results obtained by these methods used on some seaweed samples.

Table 1

Polyuronic Acid Analysis of the Fractions which are not Included in the Titrimetric Method

All figures expressed as g alginic acid per 100 g unextracted seaweed sample (dry matter).

	0·2*N* H_2SO_4 extract	Na_2CO_3– extrd. powder	Filtrates after prec. of Ca-alg.	Titrimetric method	Sum	Decarboxylation method
Alaria esculenta, Reine, 3/5–1951	1·8	3·7	0·7	31·3	37·5	38·2
Laminaria saccharina, old fronds. Reine, 9/3–1951	2·8	1·7	1·8	23·1	29·4	29·8
Laminaria hyperborea, stipes. 27/9–1950	1·3	3·5	1·4	26·8	33·0	33·6
Laminaria digitata stipes. Reine, 7/3–1951	2·4	4·4	0·5	37·5	44·8	45·5

Columns 4 and 6 show large and significant difference. Checked on pure alginic acid the two methods gave identical results. There could then be only three places to look for the origin of the observed differences: in the pre-extract, as pre-extraction is a part of the titrimetric method only; in the extracted sample as this is filtered off during the titrimetric procedure; and in the filtrates that were separated from the alginic acid during the titrimetric analysis.

These different sources were investigated for content of any material giving carbon dioxide by treatment with hot HCl, after the removal of carbonates.

Considerable amounts of carbon dioxide were obtained from these fractions as shown in column 1, 2 and 3 of Table 1.

It is very likely that the filtrates (column 3) contained small amounts of alginic acid which in the form of small particles had passed through the filter crucibles. CAMERON, ROSS and PERCIVAL introduced a rather arbitrary crucible constant to allow for this in their procedure. It was also noted that from the filtrates small amounts of alginates precipitated, and could be titrated as usual after a second filtration and a liberation of the acid.

Also the larger part of the carbon dioxide of the residual sample came from alginic acid, as introduction of double extraction in the usual titrimetric analysis resulted in higher values for the alginic acid content.

But the preextract (column 1) can hardly contain any alginic acid. Many non-uronic-acid carbohydrates give off small amounts of carbon dioxide by treatment with strong mineral acids at elevated temperatures, and laminarin, mannitol and similar compounds that are present in the pre-extract are expected to give some carbon dioxide under the conditions used.

It should be pointed out here that the titrimetric value and the amounts of alginic acid estimated in the other three fractions add up to give values very close to those obtained by the decarboxylation procedure.

From this investigation it is clear that the titrimetric method as used here gives too low values, mainly caused by incomplete extraction and filtration losses. Incomplete extraction will also influence the colorimetric method.

On the other hand, the decarboxylation procedure followed will give too high values, mainly because of easily extractable, non-uronic acid compounds that give off carbon dioxide during the decarboxylation of the alginic acid.

Removal of these interfering substances by pre-extraction with dilute sulphuric acid was therefore introduced, and the decarboxylation method was modified to form a very rapid and reliable analysis for alginic acid.

Fig. 1 shows the final apparatus. A detailed description of the apparatus and the procedure is given in a special report from the Norwegian Institute of Seaweed Research (Report No. 12), by ARNE JENSEN, IVAR SUNDE and ARNE HAUG.

The pre-extraction and decarboxylation of the sample is carried out in a sealed glass tube of special design as shown. Ten hours at

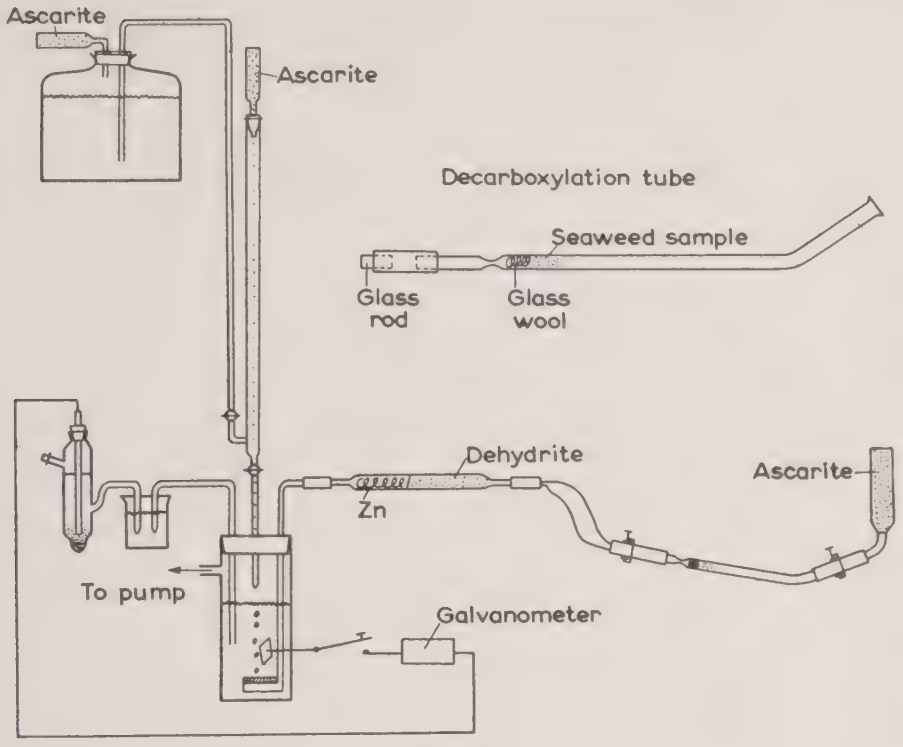


FIG. 1.

115° C in 19% HCl gives a complete decarboxylation. After cooling the tube is inserted into the apparatus, the tips broken and the carbon dioxide carried to the titration chamber by air that has been previously freed from carbon dioxide. A zinc wool filter takes care of any HCl that may be carried over from the decarboxylation tube.

The carbon dioxide is determined according to a method worked out by OELSON and collaborators (*Angew. Chem.*, **63,** 557 (1951)). The carbon dioxide is precipitated as carbonate from a very dilute solution of barium hydroxide. The ion concentration of the solution is thereby markedly changed, as observed by measuring the potential difference between a platinum electrode in the solution and a standard calomel electrode. By adding more barium hydroxide as the carbon dioxide is absorbed, the initial conditions are restored, and the amount of barium hydroxide used gives a direct measure of the carbon dioxide introduced into the titration vessel. The barium hydroxide solution and the whole apparatus is

standardized against known amounts of carbon dioxide run through the apparatus.

The method is fast, reliable, and has the advantage of being based upon a quantitative reaction.

We can add that very recent investigations of the pre-extract for the carbon dioxide-producing compounds there, led to the isolation of considerable amounts of nitrates, and it was shown that nitrate in the presence of a strong acid could oxidise organic material at elevated temperatures to carbon dioxide, the nitrate itself being reduced to nitrite.

DISCUSSION

Mr. FLOOD asked Mr. JENSEN if he had ever had any difficulties with the concentrated acid at the high temperatures used during the decarboxylation, to which Mr. JENSEN answered that this point had not caused any trouble. Mr. FLOOD further wondered whether in the very dry gas hydrogen chloride was completely absorbed by the zinc. Mr. JENSEN replied that as the method gave reproducible results and the zinc was slowly used up from one end, he felt sure that the absorption was complete.

Dr. ANDERSEN, Germany, wanted to know if the method had been tested on any pure compounds. Mr. JENSEN answered that the method was standardised against pure alginic acid which was determined titrimetrically by a special procedure. To a question from Dr. ANDERSEN about the molecular weight used in the calculations, Mr. JENSEN stated that he had used 176. Dr. SØRENSEN pointed out that it had been clearly shown by several investigators that only one mol of carbon dioxide was given off from uronic acids under proper conditions.

THE DETECTION OF SMALL QUANTITIES OF ALGINATES

By R. H. McDowell (England)

Although many tests have been proposed from time to time for the detection of small quantities of alginates, none of them is entirely specific. The best known tests make use of breakdown products of alginic acid in concentrated acid solutions to give certain colour reactions. The quantitative methods of Ross and Percival, using carbazole, and of Brown and Hayes, using orcinol are adaptations of procedures which can be used for many different uronic acid compounds. They are very useful for determining small amounts of alginates provided that other substances, which give similar colour reactions, are absent; careful separation from interfering substances is necessary. The ferric sulphuric acid test of Schroeder and Racicot is the most nearly specific we have met, but somewhat similar colours are obtained with many gums, and it is not very satisfactory in showing whether an alginate is present in a mixture of gums. This is a serious disadvantage as alginates are now often used in small quantities mixed with other colloids. In fruit products, pectates, which give a similar colour, are almost always present. An additional test for alginates which can be used in the presence of other gums is therefore very much to be desired.

A characteristic reaction of alginates is their precipitation by calcium salts; this is widely used in the analysis of moderately large amounts of alginates. It is also one method used in separating small amounts of alginates from other substances, and it therefore seemed worthwhile to examine the calcium precipitation reaction as a specific test. Calcium alginate is extremely insoluble in the presence of an excess of calcium ions, so alginate can be precipitated from very dilute solutions, but in such solutions the precipitate is very highly swollen and is not easily seen. The development of calcium precipitation as a test for small quantities of alginates has therefore involved :

(*a*) Rendering the precipitate visible.
(*b*) Finding conditions in which other substances do not interfere.

We have found that a suitable dye can be used to make traces of calcium alginate visible. The use of basic dye-stuffs for the determination of negatively charged colloids in sugar solutions was suggested by Badollet and Paine nearly 30 years ago. (In this connection the word colloid means disperse material having a much greater particle size than the large molecules of alginates and other polymers.)

Night Blue, (colour index 731, hydrochloride of tetra ethyl p-tolyl triamine diphenyl naphthol carbinol anhydride) was found the most satisfactory of the commercially available dyestuffs in the determination of sugar colloids, and we have found that it is strongly absorbed on the flocculent precipitate obtained by adding a soluble calcium salt to a dilute solution of an alginate. The flocs then take on a bright blue colour, and if the amount of dyestuff is suitably chosen the liquid becomes almost colourless.

The method is most usefully employed with alginate solutions of a concentration about 1 in 10,000. The test can be made in one drop of solution and examining it under magnification, but it is generally convenient to use about 5 ml. of solution. To 5 ml. of solution is added 0·25 ml. (5 drops) of a solution of 1 part of Night Blue in 4,000 of 50% ethyl alcohol, and then 0·1 ml. (2 drops) of a 10% solution of calcium chloride in water. The mixture is well agitated, and if alginate is present in a concentration of more than about 1 in 20,000 characteristic blue flocs will appear within a minute. 1 part of alginate in 100,000 of water can be detected, but the flocs will take longer, say up to 10 minutes, to become visible. Some turbulence in the solution is necessary for floc formation, so that it can be speeded up to some extent by agitation. The test is effective for alginates from different species of seaweed, and for alginates of the highest and lowest degrees of polymerisation available.

None of the common colloidal substances used in industry except pectates give a similar reaction, and alginates can be detected in the presence of much larger amounts of many of them. For example we have detected an alginate in the presence of gum arabic, gum tragacanth, locust bean gum, various cellulose ethers,

carrageen and gelatine without any special treatment of the solution. Magnesium pectate, unlike magnesium alginate is insoluble and pectates can be removed by precipitation with magnesium salts.

Certain conditions are necessary to obtain unambiguous results. Apart from the necessity of removing other materials which are precipitated by calcium ions, the solution should be free from suspended matter, and should not have a high viscosity. Insoluble matter, for example clay or ungelatinised starch granules will absorb the Night Blue, giving a coloured precipitate in a lighter coloured liquid. Although the appearance of such a coloured precipitate is different from that of the calcium alginate flocs, and can easily be distinguished from it, its presence will obscure a small amount of precipitated calcium alginate.

Fortunately, however, even very dilute alginate solutions can be filtered through ordinary filter papers, and kiesselguhr and cellulose pulp can be used as filter aids without appreciable loss by adsorption of the alginate, so that clear solutions can generally be obtained.

If the solution used has a high viscosity, growth of the flocs is extremely slow, and they may not become large enough to be visible. A large amount of another high polymer mixed with a small amount of alginate can therefore cause trouble, but even with materials giving the highest viscosities, such as for example a high viscosity carboxymethyl cellulose it is possible to detect 1 part of alginate in 10 of the other material. Generally a very much smaller proportion can be detected without need for separation.

Special procedures are available for some mixtures. With starch for example, it is convenient to degrade the starch with an enzyme (e.g. Nervanase) and then to filter the solution before making the test.

In a number of commercial applications the amount of alginate is less than 1/100 of the total solids present, sometimes as little as 1/1000. It is in these cases that the need for new methods of detecting the alginate is wanted, particularly when only small samples are available. Somewhat different preliminary treatments are required according to the product being examined, but it is of course expected that the analyst will have at least a general idea of the major materials present.

Properties of the alginate found particularly useful in separating it from other materials are the solubility of its magnesium salt in water, and the insolubility of the free acid.

When an alginate is present in a mixture in very small amounts it is quite likely that it will be an insoluble form, combined with calcium or heavy metals, and it is generally more satisfactory to keep it in an insoluble state in the earlier stages of separation, and to dissolve out other materials, than it is to work with the alginate in solution. It is often convenient to aim at converting the alginate to alginic acid, contaminated with as little other material as possible, and then to bring it into solution by adding a suspension of magnesium oxide, and to filter the solution before testing. The magnesium oxide makes the filtrate alkaline, and it must be neutralised before adding the calcium chloride. Acetic acid is convenient for this. If the alginate is highly degraded the acid is somewhat soluble in water, and to avoid loss in the acid stage it is preferable to work with a 50% alcohol solution to avoid loss. It is important to remove alkaline earths and heavy metals; with sufficient stages of treatment with acid this should cause no difficulty.

The following examples are given to illustrate various methods, which are only a few of the many that might be used.

In some cases a rather simple procedure can be used. For example with soaps all that is necessary is to dissolve the sample, precipitate the fatty acids by adding a solution of magnesium acetate, filter, and test the filtrate with Night Blue and calcium chloride. A test on a sample of 1 g of soap containing 1 mg of sodium alginate gave a positive result by this method.

Among foodstuffs, those free from fats and proteins are the easiest to deal with. Fruit products, such as jams and fruit drinks, and jelly sweets of various types come in this category. If the product is a solid it is first mixed with two or three times its weight of water and heated in the water bath for a few minutes to dissolve the sugar; a liquid can be treated without other preparation, but if very viscous is best diluted with a little water. To 20 ml. of the solution is added 1 ml. concentrated hydrochloric acid, followed by 20 ml. of ethyl alcohol. After thorough mixing the material is allowed to stand for 10 minutes, and the insoluble matter separated by filtration or on the centrifuge. The solids are then mixed with 20 ml. of 50% alcohol and again separated; if they are very bulky this

treatment is repeated. They are then mixed with 10 ml. water and boiled to remove alcohol. After cooling, 0·5 g calcium free magnesium oxide is mixed in, and after standing for 5 minutes the solution is filtered. The solution should be distinctly alkaline, as shown by a brown colour developing on adding the Night Blue. If this is not the case the filtrate should be remixed with the solids and more magnesium oxide. To 5 ml. of the alkaline filtrate is added 5 drops of the 1/4000 Night Blue solution, and 1% acetic acid is then added drop by drop until the solution becomes bright blue. Two drops of 10% calcium chloride solution are then mixed in, and blue flocs form if an alginate is present.

Probably the largest amount of alginates used in foodstuffs goes into ice cream, and to get a satisfactory test the fat and the greater part of the protein must be removed from the alginate. Various methods have been proposed, all of them rather time consuming. The following method which follows the lines of SCHROEDER and RACICOT has given satisfactory results, but further work is wanted to simplify it. To about 5 g of ice cream 10% acetic acid is added to bring the *p*H to about 3·5. An equal volume of alcohol is then added, and after well mixing the material is centrifuged. A mixture of protein, with any alginate present goes to the bottom of the tube, and a clear layer with some of the fat on top can be removed. A mixture of 1 part concentrated hydrochloric acid and 9 parts alcohol is then mixed in, in place of the liquid removed, and the material centrifuged again. This is repeated, and then the solids which will have decreased in volume are twice mixed with alcohol and separated on the centrifuge. The solids are then collected on a filter paper and pressed as dry as possible and fat removed with hot ether. The fat free solids are dried, mixed with 10 ml. water and 0·2 g magnesium oxide, allowed to stand 5 min. and filtered. The Night Blue, acetic acid and calcium chloride are added as before.

This method has also been found satisfactory for detecting an alginate in processed cheese.

A rather different method has been used to detect alginates in paper coatings. Here a small amount of alginate may be alone in the coating, or it may be mixed with larger amounts of starch, china clay or other materials. In any case it is likely to be present as the insoluble aluminium salt, as a result of reactions with alum in the base paper. To test for the presence of alginate, a piece of the paper,

about 50 cm^2 in area, was folded so that it could be easily inserted in a boiling tube, and was soaked for 15 min. in 20 ml. of a mixture of equal parts of ethyl alcohol and 2*N* hydrochloric acid. The spirit was poured off and replaced by distilled water. After 10 minutes the water was poured off and replaced by fresh water. This was repeated twice, and it was found that with care none of the insoluble matter was removed from the paper. 10 ml. of water and 0·2 g magnesium oxide were then added and the paper broken up in the mixture. After standing for 10 min. the mixture was filtered and the Night Blue and calcium chloride test distinguished between papers with and without an alginate present. The papers examined were sulphite papers coated for printing; with other papers modified methods might be required.

Other procedures need to be developed or existing methods modified in the examination of other products, but the purpose of this paper has been to show how the new test can be useful, perhaps as an addition to other tests in determining whether an alginate is present or not. By comparison with known amounts, a rough quantitative measure of the alginate present can be made.

If the precipitated alginate is wanted for a confirmatory test, it can be obtained as alginic acid free from Night Blue by mixing with alcohol acidified with hydrochloric acid, and centrifuged off.

In the course of the tests the use of some other metal salts in precipitating alginates in the presence of Night Blue has been noted. Precipitates similar to those with calcium are given by zinc, copper, aluminium, lead and thorium salts, but they are not specific for alginates. Possibly some of these reactions could be developed for use with other colloids. For example, blue precipitates very similar to the calcium alginate-Night Blue flocs are given with carboxymethyl cellulose and aluminium salts, pectin and copper salts, carrageen and benzidine hydrochloride. These tests are in general not specific for one colloid, but only pectates give a similar precipitate with magnesium salts.

DISCUSSION

Dr. Woodward, Scotland, wanted to know if Mr. McDowell had tried to base a quantitative determination of alginate on his findings. Mr. McDowell replied that further studies on that point had not been done but one might get a rough idea by comparing with results obtained from

solutions containing known amounts of alginate. Dr. ANDERSEN, Germany, pointed out that even in the presence of magnesium hydroxide the bifunctional dyes such as Acridine Orange might be used. This would not require the presence of calcium, and would be a further simplification of the method.

Section IV
MICROBIOLOGY

DECOMPOSITION OF LAMINARIN BY MICRO-ORGANISMS

By C. G. C. Chesters, M. Turner, and A. Apinis (England)

Read by C. G. C. Chesters

Decomposition of laminarin by micro-organisms was first reported by the present team of workers some five years ago. Barry (1941) isolated a laminarinase from the edible snail, and Dillon and O'Colla (1950 and 1951) reported this enzyme from oats, barley, potato and hyacinth. Dillon and O'Tauma (1935) showed that wheat-amylase could hydrolyse laminarin.

Seaweeds decaying either in seawater or on shore sustain a wide range of micro-organisms and some of these can degrade laminarin and alginates. In comparison with the diversity of the flora, relatively few organisms possess such powers and these vary in their ability to attack laminarin and alginates, both with respect to temperature and to the source of nitrogen available. This account attempts to summarise the present stage of our investigations.

Ability to decompose laminarin and alginates can be demonstrated by plating out dilutions in normal saline of the substrate under investigation on agar containing insoluble laminarin and suspended calcium alginate. Activity of dispersed colonies is shown by a hyaline zone round the organisms well defined against the opalescent background of the agar.

Using this technique the following organisms have been shown to be capable of decomposing laminarin.

Fucus vesiculosus

At 2° to 5° C

Achromobacteriaceae

Strain 6 and 17, from sea water: also decomposing cellulose and strain 6 decomposing starch in addition.

Strains 12 and 20, from fresh *Fucus vesiculosus*.

Strain 14, from decomposing *Fucus vesiculosus*.

Coryne bacterium tumescens, strain 10, from fresh *Fucus vesiculosus*.

In this series active laminarin decomposition only occurs at low temperatures and is not shown by the organisms even at normal laboratory temperature.

At room temperature 18° to 20° C

Nocardia citrea (Strains 1 and 13), from intertidal sand: strain 1 also decomposes starch, cellulose and calcium pectate. Species of *Arthrobacter* (strains 55 and 56), from decomposing *Laminaria digitata*.

The *Nocardia* strains also degrade calcium alginate and are more active against laminarin than the species of *Arthrobacter*.

At 30° C

A species of *Arthrobacter*, strain 45, from decomposing *Fucus serratus*.

Bacillus coagulans, strain 3, from decomposing *Laminaria digitata*.

B. pumilis, strain 21, from sea water.

B. cereus, strain 4, from decomposing *Fucus serratus*.

At all temperatures up to 30° C

Corynebacterium tumescens, strain 34, from laboratory air.

Corynebacterium fimi, strain 18, from fresh *Fucus vesiculosus*.

Bacillus megaterium, strain 54, from decomposing *Laminaria digitata*.

B. polymyxa, strain 33, from laboratory air.

Corynebacterium fimi and *Bacillus polymyxa* are two of the most active laminarin decomposing organisms so far isolated. The former also actively degrades calcium alginate, cellulose and calcium pectate.

Strains of Streptomyces isolated from decomposing brown and red seaweeds, from intertidal sands and organic muds, from salt marsh soils and from garden soils possess a high degree of activity against laminarin. So far final specific identification has not been possible, but pure culture studies and isolation of the laminarinase has shown that these organisms produce this enzyme more actively than bacteria and do so under a wider range of physical conditions and of medium composition. Strain IX, which produces a white colony on potato dextrose agar, with a brown colour in the medium

immediately below the colony, vigorously degrades calcium alginate. This is the only strain from the eighteen active against laminarin which can attack calcium alginate, all other strains degrade sodium alginate to a greater or lesser extent. All strains decompose starch and a few (IV, V, VI, IX, XI, XII, XIII) hydrolyse calcium pectate.

Laminarinase has been isolated by alcohol precipitation of culture liquors on which bacteria and actinomycetes had been growing for from 4 to 5 weeks. In this time, and at appropriate temperatures, active strains decompose 2% insoluble laminarin dispersed in culture fluid. The enzyme has been purified by dissolving in distilled water and reprecipitating with alcohol. Assay of activity has been carried out in penicillin cylinders on 2% laminarin agar when the area of clearing round the cylinder gives a measure of the activity of the extract. The following table gives some typical results:

	Zone of hydrolysis Average of five tests
Corynebacterium tumescens (34)	27 mm
Streptomyces IX	17 mm
,, (purified)	14 mm

The mycelium of Streptomyces retains a quantity of enzyme at filtration and the full effectiveness of the actinomycetes can only be gauged by pressing the mycelium to remove all adhering culture fluid.

An alginase has been isolated from Streptomyces IX by extracting the mycelium, filtered from liquid cultures, with 15% ethyl alcohol, precipitating with alcohol giving a concentration of 60% in the liquor, and washing the collected precipitate with 60% alcohol. The assays of this precipitate when dissolved in distilled water gave an average clear zone of 21 mm after 48 hours incubation at 28° C. This extract was very active against sodium alginate, completely degrading a 1% solution in 24 hours at 28° C.

Study of the stages in laminarin hydrolysis has been commenced but is not sufficiently advanced to indicate the specific processes involved.

More organisms possess the power to produce laminarinase than to produce alginase.

As well as bacteria and actinomycetes, fungi have been demon-

strated to possess laminarinase but not alginase. Such fungi are not necessarily isolated from decomposing seaweeds, and many common soil moulds have a varying degree of activity.

DISCUSSION

Dr. Young asked Dr. Chesters whether he had tested the specificity of the enzymes decomposing the alginic acid. Dr. Chesters answered that the action in general was limited to alginic acid, calcium pectate being untouched. There was some evidence for attack on calcium pectate by certain enzymes, especially by those from *Bacillus coagulans*. Apart from that observation he had not investigated the specifity of the enzymes concerned.

Dr. Isaac wondered if any of the active organisms occurred in soil, to which Dr. Chesters answered that practically all of these organisms could be obtained from soil. Older investigations had indicated that the microorganisms attacking alginic acid were new species of bacteria but Dr. Chesters was convinced that they were not. They belonged to species of bacteria which are very difficult to identify. It had taken 3 years of hard work to characterise the few species shown in his tables. He was convinced that the species identified were not confined to the sea and seaweed but were of universal occurrence.

Dr. Andersen inquired whether any thermophile bacteria had been identified. Dr. Chesters replied that 4 species might be said to have a slight thermophilic action because they could only attack laminarin at 30° C. Below that temperature they could not decompose this compound. The heating of the weed was produced by certain bacteria which were isolated, but none of the true thermophiles had been shown to attack either alginic acid or laminarin.

Dr. Aleem asked Dr. Chesters if he knew anything about the so-called black rot which was reported to be a bacterial disease that attacked the kelp in California. Dr. Chesters replied that he had no experience with this disease in his country.

BACTERIAL ENZYMES IN THE IDENTIFICATION OF AGAR AND CARRAGEENIN IN MARINE ALGAE

By W. Yaphe and Beverley Baxter (Canada)

Read by E. Gordon Young

ABSTRACT

Bacteria which hydrolysed agar and carrageenin were isolated from sea water and marine algae. Isolates were grown in media containing agar or carrageenin as sole source of carbon. Cell-free filtrate from the medium was used as the enzyme solution. Activity of the agarase and carrageeninase was specific for their respective polysaccharides. The enzymes were used to identify agar and carrageenin in the extracts prepared from a number of red algae.

Optimum conditions for activity of the agarase were at *p*H 6·0, 40° C, and 0·4% agar. The enzyme hydrolysed commercial agar and extracts from *Gelidium cartilagineum*, *Pterocladia sp.*, *Gracilaria confervoides* and *Ahnfeltia plicata*.

The carrageeninase was assayed at *p*H 7·0, 25° C, and 0·4% carrageenin. Activity was specific for the κ fraction. Extracts from *Chondrus crispus*, *Gigartina stellata*, *Furcellaria fastigiata*, *Gigartina acicularis*, *Iridophycus sp.* and *Hypnea musciformis* were hydrolysed completely or in part.

The results suggest that the κ fraction of carrageenin is present in a large number of red algae.

Section V

BOTANICAL STUDIES OF SEAWEEDS

A QUANTITATIVE STUDY OF THE BENTHIC COMMUNITIES INHABITING THE KELP BEDS OFF THE CALIFORNIA COAST, WITH A SELF-CONTAINED DIVING APPARATUS

By Anwar Abdel Aleem (Egypt)

With a Cousteau-Gagnan Aqualung and a heavy-duty diving suit, the sea bottom at La Jolla, California, was explored during spring in an attempt to assess the standing crop of the benthic animals and plants living in association with the giant kelp, *Macrocystis pyrifera*. This method of investigation ensures free movement under water and enables one to make more extensive observations of the submerged communities than by dredging or by diving with a helmet.

The visibility at the bottom is largely limited by depth, density of overlying kelp, turbulence and amount of suspended matter in the water. Thus on clear days at a depth of 20 m, the visibility ranges between 1–10 m.

Cross-sections of the sublittoral region (Fig. 1), extending over 400 m from the low tide level to the outer edge of the kelp beds,

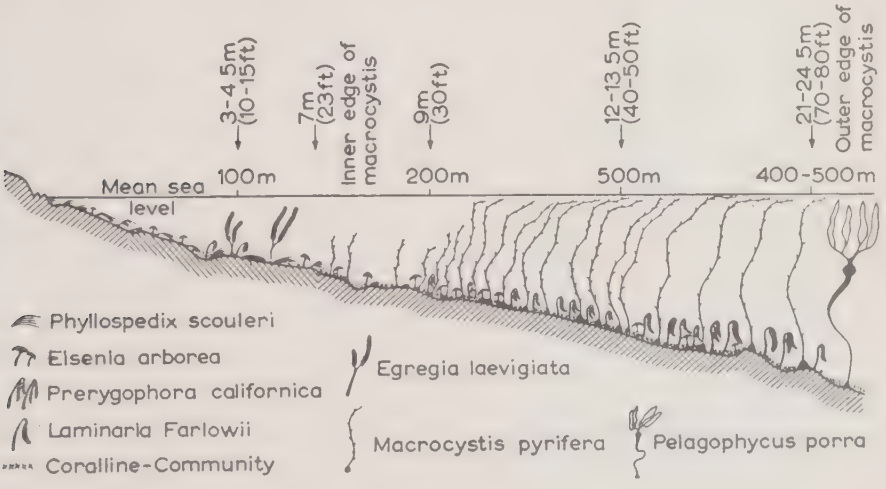


Fig. 1.

were surveyed. The organisms dwelling on the bottom were analysed quantitatively by the quadrant method. The quadrants used enclose an area of 1/4 m^2; for the larger kelp as well as for animals dispersed over wider areas, the number of organisms was counted along lines of 2 or 10 m in different directions.

The results of this survey reveal a zonal distribution of the chief submerged plants and animals, which seems to be controlled chiefly by light and temperature.

Thus, on the exposed rocks below mean low water spring tide, a community dominated by the sea-grass *Phyllospadix scouleri* flourishes best under surf conditions and extends down to a depth of ca. 7 m, covering 80 to 100% of the rock surface. Epiphytes on *Phyllospadix* consist largely of *Porphyra naiadum var. australis*, *Melobesia mediocris* and *Ectocarpus granulosus*, while *Callithamnion californicum* and *Ceramium pacificum* occur as secondary epiphytes. This community is poor in animals and apart from *Membranipora*, *Caprella* and hydroids, and a few small snails, the gross weight obtained in the quadrants is due mainly to the sea-grass and amounts to an average value of 3634 g/m^2.

A *Pterygophora-Eisenia* community flourishes where the sea-grass ceases to grow, at first as widely scattered individuals intermingled with *Egregia laevigiata*, *Cystoseira osmundacea*, *Codium fragile*, *Dictyota binghami* and *Dictyopteris zonarioides*, it reaches a climax in deeper water between 10 and 20 m, where the two main Laminariales, *Pterygophora* and *Eisenia* form, together with *Laminaria farlowii*, a 'forest' under the large *Macrocystis* plants.

Macrocystis pyrifera forms extensive beds ranging in width between 200–500 m, in a depth of water from 7 to 25 m. Individuals become more crowded in the middle of the beds, with holdfasts 1 to 2 m apart. At the outer edge, *Macrocystis* becomes replaced by scattered individuals of the elk-kelp, *Pelagophycus porra* in a depth of water of 20 to 25 or 30 m.

Where *Macrocystis* grows on outcropping rocks or on boulders, it meets with competition by sea-urchins which clear whole areas of kelp; this is demonstrated by the patchy appearance of the beds at the surface. Survival of kelp plants depends largely on the enormous production of juveniles which grow on a variety of substrata including other algae and sea-grass.

The standing crop of the *Macrocystis* plants in these beds is esti-

mated to be between 25 and 40 tons/acre with an average annual yield of about 4 to 6 tons/acre (fresh wt.) as judged by observations at the bottom, on the surface, as well as by studies of aerial photograph, by weighing representative samples and by laboratory experiments on the growth rate of kelp.

The lowermost stratum of vegetation is occupied by a coralline community which forms an extensive cover under the larger kelps. *Corallina chilensis*, *C. gracilis*, *Bossea orbigniana*, *B. gardneri* and *Lithothrix aspergillus* are the chief calcified algae inhabiting this substratum. The quantity and quality of organisms inhabiting this community were found to vary with depth, thus: (1) The percentage of cover by the dominant alga decreases with depth, hence the production per unit area decreases. (2) *Bossea* preponderates over the *Corallina* beyond a depth of 15 m. These modifications are accompanied also with modification in the fauna living in association with the corallines. For example, the sea fans, red and pink abalones, the sea star *Henricia leviuscula*, together with the holothurian *Stichopus parvimensis* and the acorn-barnacle *Balanus tintinnabulum*, become noticeable at 20 to 25 m; some of these appearing for the first time at such depths.

In Table 1 a comparison is given of production of the coralline community from different depths.

Table 1. Standing Crop of the Coralline Community at Different Depths

(Average fresh wt. of plants and animals/m^2 is given; depth in m below mean sea level.)

Depth (m)	Plants (g/m^2)	Animals (g/m^2)	Total Wt. (g/m^2)
1·5	4667	125	4712
7	2490	329	2819
15	1972	392	2364
22	606	377·2	983·2

It has also been found that, while the total weight of plants decreases with depth, that of the animals tends to increase. Based upon the foregoing survey, an average figure for the standing crop of the organisms inhabiting the rocky bottom of the kelp beds, including *Macrocystis* itself, would approach 9·4 kg/m^2 of sea bottom or approximately 38 tons/acre. These figures do not include the pelagic forms.

DISCUSSION

Dr. BURROWS, England, asked how Dr. ALEEM dealt with irregularities in the surface which she had found made an enormous difference in the number of species recorded. She also asked whether the minimal area could be determined in such a way as to be of any practical use. He replied that, for the purpose of such a general survey, he avoided such places as crevices, caves etc. and confined himself to species with a convenient distribution. Also, he had found the 'micro-climate' in a community an interesting study. A small stone may have quite a different flora on its upper than on its lower surface.

THE GROWTH RINGS IN *Alaria* STIPES

By Egil Baardseth (Norway)

The zonate appearance of stipe sections of many *Laminariales* is well known. The zones have been explained by periodic changes in (1) size of cells and cell walls, (2) colour of cell walls, (3) content of cells, and (4) arrangement of cells. Some maintain that the zones represent annual increments, some not. The resemblance to annual rings in trees is obvious, but we hope to show that it will be wrong, in many cases, to put the number of rings in a cross section of Alaria stipes equal to the age in years.

The zones in *Alaria* consist of a dark, narrow part and a light-coloured, broad part. The former part will be referred to as a 'ring' in a cross-section and a 'pair of lines' in a longitudinal section. They obviously contain a greater quantity of 'fucosan vacuoles' than do the surrounding light-coloured tissue. The rings and lines are consequently easily stained by a number of 'fucosan' reagents, such as osmic acid, $FeCl_3$, benzidin-$NaNO_2$, vanillin-HCl, and triphenyl-tetrazoliumchloride.

The innermost ring or pair of lines in sections close to the base of the plant will be called No. 1. The others will be numbered successively 2, 3, etc. We will start at the base of the plant and see what happens to the lines and rings when we proceed up the stipe. The figure shows a longitudinal section through the plane of the flattened medulla and two longitudinal sections perpendicular to this plane, one cut centrally and the other tangentially.

All lines start either from the haptere regions of the stipe or from the periphery of the stipe somewhere higher up. They were never seen in the hapteres themselves. Pair of lines No. 1 are always thinner than No. 2, and may sometimes be very short, one cm. for instance. They always run continuously through the stipe, whereas lines of higher numbers may often be discontinuous, such as lines Nos. 4, 5 and 6 in the diagram. These start at the base, but after a short distance merge into the epidermis and disappear. No. 4 is seen again a short distance above, whereas Nos. 5 and 6 do not reappear until we come to the upper part of the stipe. In this particu-

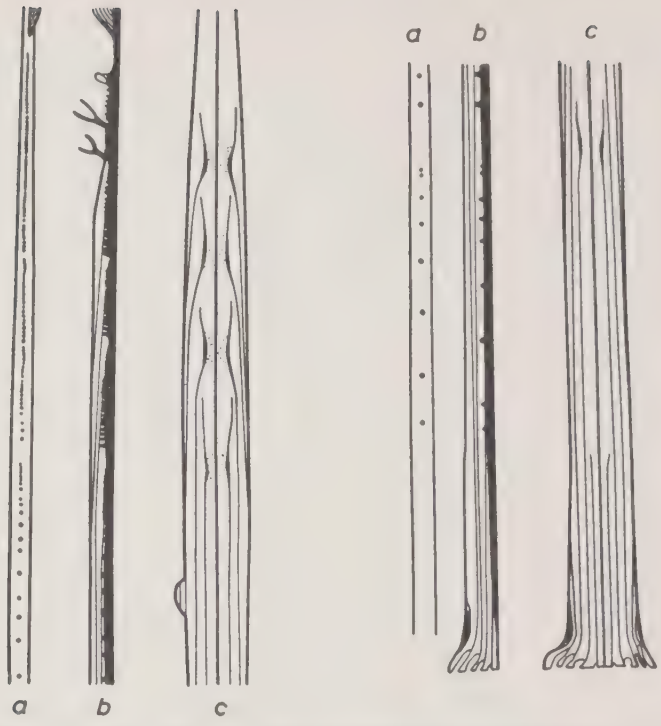


FIG. 1. (*a*), (*b*), and (*c*). Three longitudinal sections through a stipe of *Alaria esculenta*, (*a*) cut tangentially and perpendicularly to the plane of the medulla, showing sporophyll scars as dots; (*b*) cut through the plane of the medulla, showing sporophyll stipe scars as outgrowths from the medulla with existing sporophylls (most of them cut away) on top; (*c*) is cut centrally and perpendicularly to the plane of the medulla, showing the latter as a continuous line in the middle and 6 pairs of lines. Length of stipe x $\frac{1}{6}$, breadth x $\frac{1}{2}$.

lar stipe, there are no more than these 6 pairs of lines and they all exist at the base. In many other stipes, however, I have seen lines in the upper part which do not exist at the base at all. Thus I have seen a stipe with only 3 pairs of lines at the base and 7 pairs of lines altogether. The other 4 did not appear until higher up. It will be understood, then, that if we want the exact number of lines and rings, we cannot just count them in a section near the base, because some rings may not exist there at all. If we want the correct number of lines, it seems that a longitudinal section through the whole length of stipe is unavoidable.

Just before the final disappearance of a pair of lines, a number of remarkable changes are frequently seen. The lines themselves approach each other gradually until a certain point, then diverge

slightly. It often looks like a slight constriction of the lines at this point. The narrowest part of the lines is also frequently somewhat thicker than immediately above or below. If the sections are treated with vanillin-HCl, most of the tissue takes on a rosy red colour, including the lines themselves. However, a bluish tint is given to the transition zone between blade and stipe. Blue spots, but of a fainter colour, are also found at intervals lower down, always in the inner cortex inside the constricted and thickened part of the lines (dotted areas in the diagram).

In a longitudinal section through the plane of the medulla, outgrowths from the latter are frequently seen. They always sit on both edges of the medulla and cannot be anything else than remains of former sporophyll stipes which have been overgrown by secondary tissue. They once continued out in a sporophyll stipe and blade which dropped off at the end of the season and left behind these small projections of medullary tissue. An accurate sectioning thus reveals the position and number of all sporophylls produced by a plant during its whole lifetime. It will be seen from the diagram that the distance between the sporophyll scars is not constant. They are sometimes close together, sometimes widely spaced. A concentration of scars is very frequently seen at the level of, or slightly below, the constricted and thickened part of the lines.

All these four characteristics, the constricted part of the lines, the thickening, the bluish spot produced by vanillin-HCl, and the sporophyll scar concentration occur together at the same level of the stipe so often that it cannot be accidental. An explanation is called for and it seems very likely that these levels must represent old transition zones of former years left behind by the new stipe tissue of early spring. In fact, the transition zone of this year is also characterized by a concentration of sporophyll stipes and by a blue spot produced by vanillin-HCl. It also seems that the fucosan-containing part of the epidermis, later to become a line or ring, is slightly thicker than below. If this explanation is correct, one should expect the lines to stop exactly at the spots supposed to be former transition zones because the blade and midrib above the present transition zone decay and disappear at the end of the season. The lines do not stop abruptly like this, however, and their continuation above the former transition zones needs some supplementary explanation. We may, for instance, assume that this

upper part of the lines may be produced either by the new transition zone of the next year or by a secondary growth in length of the upper part of the present transition zone.

There can be no doubt that there is a periodicity in the production of sporophylls, at least in Norway. A set of ripe sporophylls is present during spring and early summer. These drop off during winter and early spring and a new set is produced on top of the old ones during the months January–March. We can thus safely speak of sporophyll generations, one generation being produced each year. Each generation terminates in a concentration of small and rudimentary sporophylls which later probably remains as the scar concentration just mentioned. This means that the probable position of the sporophyll generation of former years can be determined. Each sporophyll generation can be associated with a pair of lines which means that these must be annual lines. It should be noticed that sporophyll scars are very seldom found alongside the first pair of lines. This accords with the fact that first year plants only occasionally carry sporophylls.

Unfortunately, some sections are not always easily interpretable. A line may sometimes divide into two equally strong lines. Quite often, especially in thick stipes, faint lines may run for a shorter or longer distance in between strong lines. The former lines cannot be brought in connection with a sporophyll generation. Consequently, if we accept the yearly appearance of one strong line together with one sporophyll generation, the faint lines must be 'false' annual lines, i.e. two rings or lines, one strong and the other faint, may be produced during one year.

In conclusion, we may attempt an outline of the probable life-history of *Alaria* plants in Norway: New plants arise in April. They produce a short blade and, in some cases, a first sporophyll generation during the same spring and summer months. The blade drops off during late summer and autumn, whereas the sporophylls are shed somewhat later in the winter. A ring is not visible in first year plants. During the winter and spring months of the following year, a new blade and a new sporophyll generation are produced, at the same time as the first ring or pair of lines is made visible by the secondary growth in thickness and length of the stipe. The blade reaches its maximum length in May–June, disappears during the summer and autumn months. The further development follows

the same pattern in the following years, with the exception that some parts of the stipe may stop the secondary growth altogether, and some parts may produce two rings a year.

DISCUSSION

Dr. HAXO, U.S.A., wanted to know whether Dr. BAARDSETH had found other kelps with similar rings. The answer was that the rings of *Laminaria hyperborea* may perhaps be used in an age determination, but he was not quite sure because he had seen two types of rings.

Dr. CONWAY, England, remarked that the sporophyll distribution must have played an important part in Dr. BAARDSETH's investigations and asked if there are other species which could be dealt with in a similar way. Dr. BAARDSETH thought that the Californian *Pterygophora* is such a species.

Dr. ALEEM asked whether the rings were conducting elements, but Dr. BAARDSETH said that there was no other difference between the cells of the rings and those of the outer cortex elsewhere than a concentration of fucosan vesicles in the rings. Dr. ALEEM also wanted to know how the rings were formed and Dr. BAARDSETH suggested as a possible explanation that the fucosan-containing cells were produced mainly in the summer, and that these were overgrown by a light-coloured tissue in the spring.

Dr. ISAAC commented upon the relative absence of change in temperature of the cold water in South Africa and the absence of a ringed structure in *Laminaria pallida* and *Ecklonia maxima*.

Dr. MOSS, England, asked how old Alaria could be and Dr. BAARDSETH answered 7 years as a maximum.

Mr. RICHARDSON, Scotland, said it is possible to measure the area between consecutive rings in order to assess the growth rate in *Laminaria hyperborea*.

OBSERVATIONS ON THE TIME OF REPRODUCTION AND THE RATE OF GROWTH OF CERTAIN *Fucaceae*

By Helen Blackler (Scotland)

When the periodicity of the reproduction of many algae is studied, it is found that the time of reproduction of certain species varies in different latitudes and also the rate of growth of vegetative and reproductive parts; though there are not many places where such studies have been made, nor many species so investigated.

Records of the time of reproduction and the rate of growth of certain Fucaceae have been made by various workers, at Roscoff, Plymouth, Aberystwyth, Port Erin in the Isle of Man, St. Andrews, Scotland, the Faeroes and in Norway.

Ascophyllum nodosum, *Fucus serratus*, *Fucus vesiculosus*, and *Himanthalia elongata* have been investigated in some of the above mentioned places for the time of reproduction and the rate of growth.

In *Ascophyllum nodosum* there is a change from a winter-spring period of reproduction in France to a spring-summer period in the British Isles and the Faeroes.

At Roscoff according to Hamel (1931) the receptacles appear in June–July and are ripe from November–February, being shed from March–May whereas in St. Andrews they appear in August and develop through the winter. The developing oogonia can be seen from October onwards, but are not ripe until February. The fertile period lasts until June, and the spent receptacles are shed during June and July. Thus in Roscoff the receptacles take four to five months to develop and six months in St. Andrews thus indicating a slower growth rate than in France. According to Sundene (1953) in the Oslofjord development is similar to that in St. Andrews, but a little slower. Receptacles being well formed by the middle of August, with unripe oogonia in October which are not ripe till the end of March, the plants are fertile till June.

Fucus serratus and *Fucus vesiculosus* have been investigated at

Roscoff by LEMOINE (1913) and by KNIGHT and PARKE (1950) at Plymouth and in the Isle of Man and on the west coast of Scotland in Argyllshire.

In *Fucus vesiculosus* in France, according to HAMEL (1931), the receptacles appear in December or January and reproduction occurs in the spring while from June onwards the receptacles begin to decay and become rare though they may persist through the summer. At Plymouth, KNIGHT and PARKE found that the receptacles are ripe and liberating gametes from April till July. In the Isle of Man gametes are liberated in March with a peak period in May, but can continue to be liberated in a small percentage of the population until September.

Fucus serratus

In France fruits in the spring and in summer, the receptacles decay though some may persist till the winter. In Plymouth the fruiting period is from July until December, in Aberystwyth, from August until February and in the Isle of Man from October until the end of February (autumn–spring) while in St. Andrews reproduction is most prolific during the autumn and winter and spring until May though many plants reproduce throughout the year. Thus there is a change from a spring-summer fruiting period in France to summer-winter in Plymouth and autumn-spring in the Isle of Man and more or less throughout the year in St. Andrews. LEMOINE (1913) and KNIGHT and PARKE (1950) also investigated the growth rate of *Fucus serratus* and *vesiculosus*. LEMOINE found that the growth rate of *Fucus serratus* could be at the rate of 5 cm per month while KNIGHT and PARKE found that the growth rate for this species varied according to the position of the plants on the shore with regard to shelter and was more rapid in the north than in the south though slower everywhere than in France. They found that the normal average growth rate for the first three years of a plant of *Fucus serratus* is:

0·49 cm on the Devon coast per week
0·68 cm on the Manx coast per week
0·85 cm on the Argyll coast per week

in contrast to 1·2 cm per week at Roscoff.

The growth rate of *Fucus vesiculosus* is slower both in France and Britain. LEMOINE found it to be from 0·62–0·82 cm per week while

KNIGHT and PARKE found the average rate of elongation per week to be as follows:

0·48 cm on the Devon coast per week
0·45 cm on the Manx coast per week
0·68 cm on the Argyll coast per week.

The growth rate of *Himanthalia elongata* has been observed at Roscoff by LEMOINE (1913) and on the Manx coast by GIBB (1937) and by the author at St. Andrews.

At Roscoff *Himanthalia* completes its development in one year, the receptacles being fully formed and liberating ripe gametes in the summer, and in the autumn the whole plant including the vegetative button is washed off the rocks. In the Isle of Man and at St. Andrews two years is required to complete the lifecycle while according to WILLE two to three years are needed in Norway. The French plants are longer than the Manx or those from St. Andrews being three metres or more in length, the Manx ones are about one to one and a half metres long on an average, while they are on an average less than a metre in St. Andrews, and WILLE gives half a metre as the size of the Norwegian plants.

Gibb found that there was a variation in the growth rate according to the position of the plants on the shore, and by careful measurement of two sets of plants month by month from low water of springs and from about a foot above low water neap tides found that the plants at low water springs grow quicker and are longer and narrower than those from just above low water neap tides.

At St. Andrews plants from similar positions have been measured month by month over several years and there is the same variation in length and breadth but the growth rate is slower than in the Isle of Man. The plants from low water springs at St. Andrews are not so long and are narrower than the Manx ones from the same station, while those just above low water neap tides are shorter and broader than those from the corresponding position in the Isle of Man.

In the latter locality initiation of the receptacles usually begins in July in the second summer from the centre of the buttons and in August to September in St. Andrews. Growth of the receptacles in both places is very slow at first until October–November. In the

spring there is a rapid acceleration of growth, particularly from March to June, which is most noticeable in the plants at low water springs. The growth curves of both the plants at low water springs and just above low water neaps in the Isle of Man and St. Andrews are very similar. The maximum length is reached by the end of June after which time the ends of the receptacles become worn away as the gametes are shed from the conceptacles. A few plants may remain through the winter and become very much worn and battered by wave action.

The growth in breadth follows the same pattern in both the Isle of Man and at St. Andrews. At the latter place it is noticeable that the increase in breadth is rather less than usual when the oogonia and antheridia are ripening from June to July, although that is a period of rapid elongation of the plants. After July the plants continue to increase in width for some time but there is a decrease in length as the ends of the plants are worn away and this is most marked at low water springs.

Cryptostomata are formed by February and development of the reproductive organs in the conceptacles occurs in March and April though they are not mature and liberated until the end of June.

The branching in both the Manx plants and those at St. Andrews occurs in the early part of the year. In the Isle of Man plants growing at low water springs show branching from November to January and from December to February at the higher level. At St. Andrews branching occurs at the same time at both levels and is complete by April.

The plants show the same growth pattern in the Isle of Man and at St. Andrews, but the growth is greater and more rapid in the Isle of Man probably due to the higher sea temperature and other environmental conditions.

REFERENCES

GIBB, DOROTHY C. (1937) Observations on *Himanthalia lorea* (L.) *Lyngb. Linn. Soc. Botany* vol. 11 (No. 337).

HAMEL, G. (1931–1939) *Phéophycées de France*, Paris.

KNIGHT, MARGERY & PARKE, MARY (1950) A Biological Study of *Fucus vesiculosus* L. and *F. serratus* L., *J. Mar. Biol. Ass. U.K.*, vol. XXIX.

LEMOINE, M. (1913) Quelques experiences sur la croissance des algues marine à Roscoff, *Bull. Inst. Oceanogr.* No. 277, Monaco.

SUNDENE, O. (1953) The Algal Vegetation of Oslofjord, *Skrifter utgitt av Det Norske Videnskaps-Akad. Oslo, Mat. Naturv. Klasse* 1953, No. 2.
WILLE, N. (1910) Der anatomische Bau bei *Himanthalia lorea*, *Jahrb. f. wiss. Bot.*, T. 47.

DISCUSSION

Dr. Moss, England, asked whether Dr. BLACKLER was sure that habitats at the different latitudes had been absolutely identical because she had found *Himanthalia elongata* of an enormous length in a narrow channel on the west coast of Argyllshire. It was growing sublittorally, but its length was due to habitat rather than to the latitude. Dr. BLACKLER answered that she had also seen *Himanthalia* plants of an enormous length in currents in Ireland. She did not think, however, that the French specimens had been swift current forms.

Dr. BURROWS, England, also stressed the importance of an exact definition of the habitat before considering a comparative growth-rate. She had found an enormous difference in the growth-rate of certain *Fucaceae* from various habitats. Thus, on the outer and inner side of the breakwater at Port Erin, she had found a difference in the growth-rate of *Fucus vesiculosus* which was very much higher than the whole range given by Dr. BLACKLER for different latitudes. Dr. BURROWS had also found large differences in the growth-rate in fronds on one and the same plant of *Ascophyllum nodosum*.

Dr. CONWAY, Scotland, remarked that she had found *Himanthalia* 2–3 m long in the North of Scotland.

Dr. ISAAC, South Africa, had made the observation in South Africa that on verticals with considerable wave action *Hypnea spicifera* grows to more than twice the length found on more protected flat surfaces.

Dr. BLACKLER finally remarked that she had found a different rate of growth at different levels of the shore.

GROWTH CONTROL IN THE *Fucaceae*

By E. M. Burrows (England)

During the course of an attempt to analyse the variation occurring within the genus *Fucus*, a study was made of the comparative anatomy of the three species *Fucus spiralis L.*, *Fucus vesiculosus L.* and *Fucus serratus L.* and forms of these growing under different ecological conditions. It was hoped that such a study might provide an additional criterion for distinguishing the species and help in the recognition of naturally occurring hybrids among them. The study was not successful from this point of view; the basic pattern of anatomy was similar for all three species and environmental conditions appeared to produce greater modifications than could be attributed to specific differences. Nevertheless, a number of facts emerged which it seemed worthwhile to record.

The material for the investigation was fixed in a chrom-acetic fixative made up in seawater, taken up to 50% alcohol, sectioned by hand and mounted in 50% glycerine. Dehydration was avoided because of the resulting disturbance to the mucilage walls of the cells. The anatomical changes taking place in the thallus, from the dividing apex to the mature base, were followed by means of transverse and longitudinal sections drawn to a fixed scale using a camera lucida. As a result, one particular feature which has been recorded in a general way in descriptions of the development of the mature thallus, when seen in plants grown under different habitat conditions, appeared to have a significance which has so far not been recognised. This feature is the extended enlargement phase, separating cell division at the apex from subsequent cell division, in the cortical region of the thallus.

The differentiation of the *Fucus* thallus from the fertilised egg and also the adult structure have been described a number of times (see Fritsch, 1947) and will not be dealt with here. Attention was given to the method of secondary thickening of the thallus as long ago as 1876 by Reinke and more recently and in more detail by Pennington (1937). Secondary thickening was found to be the result of three main processes:

1. Enlargement of the cells and especially those of the medullary region.
2. Tangential division in the cells of the inner cortex.
3. The formation of hyphal filaments which arise as outgrowths from the inner cortical cells and penetrate between the primary cells of the medulla.

From a very early stage, enlargement of the thallus is effected by an increase in the size of the cells of both cortex and medulla and by separation of the primary filaments in the medulla as a result of gelatinisation of the middle lamellae and accumulation of mucilage between them. These processes are most marked in the midrib region of the thallus; the wings remain much thinner and ultimately disappear, leaving the midrib as the stipe of the thallus.

In the present study attention was directed particularly to the enlargement and division of cells in the cortical layers. For some time after their formation from segments of the apical cell, no division takes place in the cells in these layers, but they undergo considerable enlargement. The enlargement phase is followed sooner or later by a simultaneous division in all planes in the cells of one or more layers beneath the palisade layer, giving a considerable increase in the number of cortical layers. Only very occasional divisions appear to take place in the surface layer itself and with rapid increase in diameter of the thallus as a whole, this layer is ruptured and shed, while the outer walls of the cells remaining at the surface develop considerable thickenings.

In relation to these aspects of their anatomy, interesting differences were found between forms of *F. vesiculosus* growing under sheltered and rough water conditions. The effect of rough water on the growth of *F. vesiculosus* is to reduce the growth rate so that narrow, short plants, with relatively thick stipes, are developed; they lack the usual air vesicles. The two ends of this variation range have been distinguished for some time, for rough water, as *F. vesiculosus var. evesiculosus* Cotton and for sheltered water as *F. vesiculosus var. vadorum* Aresch. Direct measurement of fronds, of approximately the same initial length, over the rapid growing period from March until the end of August, showed that the growth rate under sheltered conditions was considerably greater than that under rough water. This is illustrated in the graph in Fig. 1.

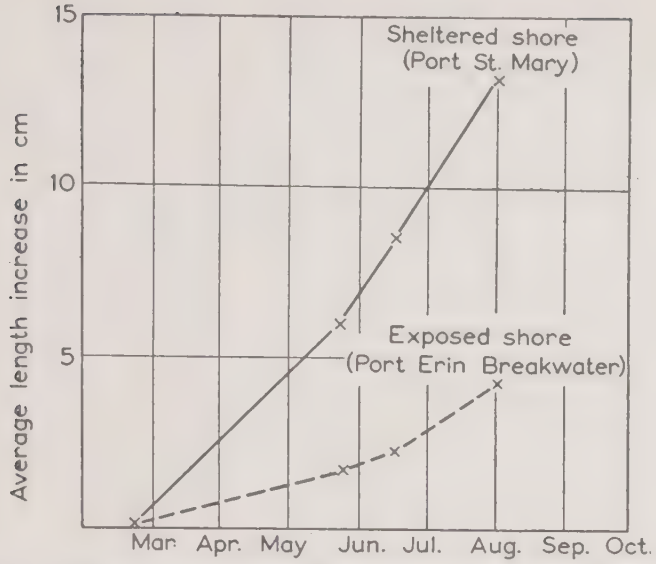


FIG. 1. Comparison, for rate of increase in frond length, between sheltered and exposed coast forms of *F. vesiculosus*.

An examination of the anatomy of *F. vesiculosus var. evesiculosus* from the outer side of the breakwater at Port Erin, Isle of Man, showed that, in comparison with plants growing under less exposed conditions at Port St. Mary, cell enlargement proceeded at a slower

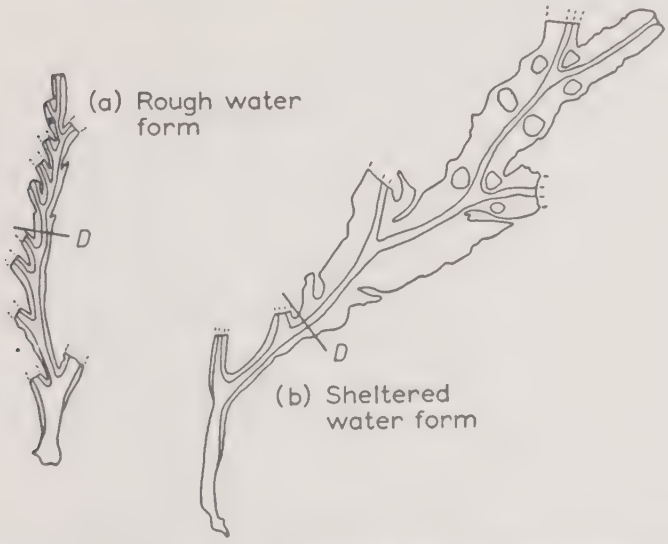


FIG. 2. Thalli of *F. vesiculosus* showing the positions of cell division (D) in the cortical layers.
(*a*) Rough water form. (*b*) Sheltered water form.

rate, and, as a result, the cell division in the outer layers appeared to take place at a shorter distance behind the apex. The positions of cortical cell division in the two plants are shown in Fig. 2. The cell division in *F. vesiculosus var. evesiculosus* took place while the cells were smaller, suggesting that the ability to divide is not dependent on the size of the cells. Since sections of plants, taken at any time of the year, show this wholesale division of cortical cells at some point in the thallus, it is apparent that it is not related to any particular change in environmental conditions. Taking into account the relative growth rates and the position in the thallus at which division occurs in the two forms of *F. vesiculosus* under discussion, there would seem the possibility that it is either the age of the cells which is important in this connection or, alternatively, the distance, in terms of the number of cells, separating them from the active apex.

The slower growth rate of *Fucus* under exposed conditions is probably the result of a reduced light supply. The plants are covered by water for longer periods and the surface of the water is almost continuously broken, thereby limiting the penetration of light. Little is yet known concerning the optimum conditions for photosynthesis in these algae in terms of light supply, availability of carbon dioxide under exposed and submerged conditions, changes of temperature resulting from exposure to air and changes due to drying of the thallus. Vallance and Coult (1951) who made analyses of the gases contained in the vesicles of *F. vesiculosus* under different conditions, suggested that vesicle formation may possibly be conditioned by the intensity of photosynthetic activity and that 'The decrease in intensity of the light incident at the surface of the plants growing in rough water, may well account for both their stunted stature as well as for their normal failure to produce vesicles.'

Further, it would appear to be the enlargement phase of growth that is adversely affected by a reduction in light supply. Cell division can take place independently of light. Experiments were carried out with the fertilised eggs of *F. vesiculosus* and *F. serratus* and it was found that they will segment in the dark and form embryos consisting of very small cells: a rhizoidal protuberance is first formed and cell division proceeds to a stage of approximately 32 cells, although there is practically no increase in the diameter of the em-

bryo beyond that of the fertilised egg from which it has arisen. If the eggs are grown in the light, a cell division stage takes place first and is followed by a stage in which division at the apex and enlargement behind the apex proceed simultaneously. The first enlargement phase therefore seems to depend on a light supply and may result from the beginning of active photosynthesis in the embryo.

Thus in the development of the *Fucus* thallus, two periods of cell division are separated by a more or less extended period of cell enlargement and the process of enlargement appears to be influenced by the external factor of light supply.

It seemed likely also that, as with higher plants, there might be apical control of cell division. A test was made to find the result of removing the tips of a number of growing fronds. Twenty-five plants were marked on the shore in January 1953; of these 13 had all apices removed while 12 were left untreated. The plants were collected in October the same year and examined anatomically. *Fucus spiralis* was used as the experimental plant for the sake of convenience, but the results can almost certainly be applied to *Fucus vesiculosus* since development follows the same pattern in both species. Only 6 plants remained on the shore at the end of the experiment: of these 5 were 'cut' and 1 'uncut' thalli. For the purpose of the immediate experiment, however, any undamaged plant of *F. spiralis* on the shore in the vicinity could be used as a control plant.

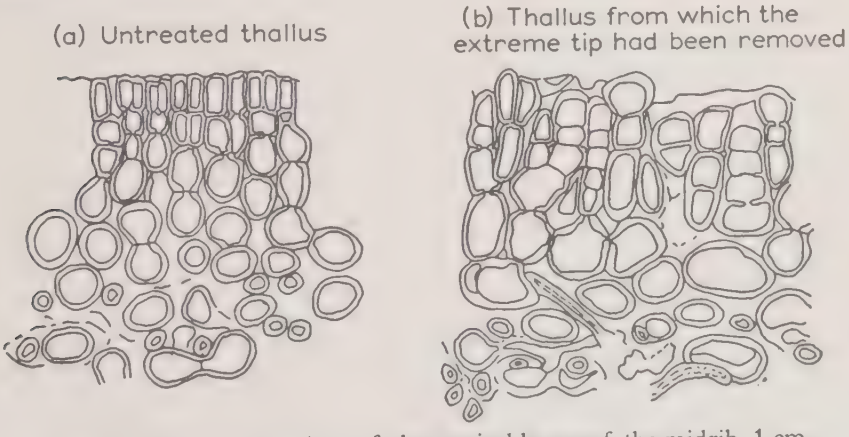


FIG. 3. Tranverse sections of the cortical layers of the midrib, 1 cm behind the apex, of thalli of *F. spiralis*.
(*a*) Untreated thallus. (*b*) Thallus from which the extreme tip had been removed.

An investigation of the anatomy of these plants showed that in thalli from which the apices had been removed, cell division had occurred over the whole of the normal enlargement region of the cortex: in uncut thalli, division occurred at some distance behind the apex. Fig. 3 shows a comparison between the situation 1 cm behind the tip in a cut (*b*) thallus and an uncut (*a*) thallus. In the cut thallus the outer cortical layers had already been lost and it was not possible, at this stage, to tell in how many of the original cortical layers division had taken place: only the innermost layer remained and was dividing. In addition to the difference in the position of cell division, the cut thalli showed other unusual features. At their apices, in some cases, new fronds were formed as proliferations and groups of proliferations appeared in various positions on the thalli, but always in the midrib region. The results indicate that there is apical control of cell division. The removal of apices stimulates the organisation of new apical cells, not only in the region of the original apical cell but also in parts of the thallus remote from the apex: the stimulus resulting in this proliferation travels in the midrib region of the frond (see Fig. 4). Apical control

Fig. 4. Plant of *F. spiralis* from which the apices had been removed 9 months previously.
p = proliferation.

of branching in the *Fucaceae* has also been demonstrated for *Ascophyllum nodosum* Le Jol., a species in which monopodial as well as dichotomous branching occurs (Burrows, 1947). For *Ascophyllum*,

cutting of the fronds resulted in a development of new lateral branches from latent apices behind the cut surface. The new branches were formed in a basipetal succession. It was also observed that plants growing under conditions of reduced salinity produced branches, both as true laterals and sometimes also as proliferations, in great numbers, even in the presence of the intact apex. The cells, and particularly their mucilage walls, absorbed quantities of water and swelled; under such conditions, the apex appeared to exert a diminishing inhibiting effect on branch formation. Thus a reduction in the salinity of the water in which the plants were growing appeared to have the same effect as the removal of the frond apices.

Auxins are almost certainly involved in these processes. Du Buy and Olsen (1937) have shown that the eggs of *Fucus* contain an auxin and, although it was not identified, it was extracted and found to be active in determining the polarity of the embryo. Van Overbeek (1940) gave figures for indoleacetic acid equivalents of auxin concentrations found in different parts of the thallus of *Fucus evanescens*. Although, on the whole, they were relatively low, some at least could be matched by concentrations found in higher plants. Davidson (1950) in an attempt to find whether externally applied concentrations of auxins produced growth responses in non-vascular plants comparable with those in vascular plants, found that the growth of vegetative thalli of *Fucus evanescens* and *Ascophyllum nodosum* in seawater could be accelerated by the application of certain concentrations of indoleacetic acid and naphthalenactic acid, and, in the case of *Fucus*, indolebutyric acid also. Maximum growth was produced in concentrations which appeared to be specific for each particular hormone. There was a suggestion in his work that the responses to the application of auxins were affected by light. Plants subjected to sunlight during application of the growth substance in seawater, turned dark and showed signs of disintegration within 2–3 days, while plants shaded by fronds from surrounding algae gave growth responses to application of auxins in low concentrations. An interpretation of any of these results at the present time would, however, be meaningless.

The type of growth response to light supply shown by these plants is of obvious importance to the larger brown algae growing in the intertidal region. The vegetation of this region is frequently subjected to denudation by the force of waves, bringing about

changes which have no parallel for land vegetation. In a fully developed zonation, the Fucoids cast considerable shade on the rock surface beneath, both at high and low tide. During the fruiting periods, large numbers of gametes are shed into the sea where fertilisation takes place and the fertilised eggs can attach themselves to the rock surface and segment, even in the absence of light, to form small embryos of about 32 cells: beyond this stage, growth in length is dependent on a light supply. Under shaded conditions growth is very slow and sporelings may remain for long periods at a very small size. The increase in light for patches of the rock surface, occasioned by the removal of adult fronds, gives a stimulus to rapid enlargement of the sporelings, thereby increasing their surface area, and growth is greatly accelerated. The removal of the adult fronds from the shore thus provides the conditions necessary for the rapid development of the sporelings on the surface beneath them.

The author wishes to thank Professor N. A. Burges, Mr. D. A. Coult and Professor J. H. Burnett for reading and criticising the manuscript.

REFERENCES

Burrows, E. M. (1947) A Biological Study of *Ascophyllum nodosum*, Ph. D. Thesis, London University.

Davidson, F. (1950) Effect of Auxins on the Growth of Marine Algae, *Amer. J. Bot.*, XXXVII, 502–510.

Du Buy, H. G. and Olsen, R. A. (1937) The Presence of Growth Regulators during the Early Development of *Fucus*, *Amer. J. Bot.*, XXIV, 609–611.

Fritsch, F. E. (1945) *The Structure and Reproduction of the Algae.* II, Cambridge.

Pennington, W. (1937) The Secondary Thickening of *Fucus*, *New Phyt.*, **36**, 267–279.

Reinke, J. (1876) Beitrage zur Kenntis der Tange, *Jahr. wiss. Bot.*, **10**, 317–382.

Vallance, K. B. and Coult, D. A. (1951) The Composition of the Gas contained in the Vesicles of *Fucus vesiculosus*, *Ann. Bot. N. S.*, XV. (No. 59), 409–416.

Van Overbeek, J. (1940a) Auxin in Marine Algae, *Pl. Phys.*, **15**, 291–299.
(1940b) Auxin in Marine Algae, *Bot. Gaz.*, **101**, 940–947.

DISCUSSION

Dr. Moss and Dr. Burrows commented on the effect of temperature and salinity on the morphological differentiation.

SEAWEEDS OF ECONOMIC IMPORTANCE IN SOUTH CHINA

By Bek-To Chiu (Hong Kong)

ABSTRACT

In China, marine algae bear the name, sea vegetables' and have been used as such by the coastal population since prehistoric times. At least 20 species can be enumerated, used as an ingredient in soup, fried or broiled dishes, or as a condiment, for its savoury flavour and for its crisp or mucilaginous texture. Sun dried or processed specimens are stocked in all grocery shops. The red forms form the main source of all GEL food.

As medicines, they are recognised as having a cooling effect in time of fever, brewed for drinking in the form of tea. The brown algae, *Sargassum fusiformis*, *S. Thunbergii* and *Laminaria japonica* have long been used for producing a cooling and blood cleansing effect, for treatment of glandular weakness, apoplexy, and for normalising blood pressure. The red algae, *Digenia simplex*, forms the basic stock for the manufacture of the local drug called TSE Ko-Tsoi, taken by adults and children alike as an anthelmintic throughout the country, and is of great economic concern.

In industry, a paste for sizing silk is obtained from the red alga, *Gloiopeltis furcata*. The sized black silk is worn by a big majority of the population, men and women alike, throughout the long summer months. The durability of the silk and the cooling effect in wearing depend largely on the quality of the paste used in sizing, obtained mostly from the tetrasporic plants. Size of inferior quality, obtained from the cystocarpic plants, is used in the industry, also of enormous concern, in converting silver to gold 'ghost paper' for use as money for the dead in the next world.

The bulk of the *Sargassum sp.*, abundant in this region, is harvested for use as fertiliser for crops or as fodder for pigs.

DISCUSSION

Mr. POWLING, U.K., expressed his gratitude to Miss CHIU for her interesting and charming lecture.

Dr. NEWTON, England, pointed out the scientific value of Miss CHIU's work and she drew attention to the *Gloiopeltis* species where certain observations indicated a possible difference in the properties of the gels from the haploid and diploid forms. She also wondered if the shortening of the two life cycles into such a very brief period would lead to an early germination of the spores. She asked Miss CHIU if she had done or knew of any work on the germination of these curious plants with haploid and diploid reproductive organs on the same thallus.

Miss CHIU answered that she had grown some of these plants in the laboratory, but she had not reached any conclusions concerning the germination yet.

Dr. ISAAC, South Africa asked for further details about the bare rocks on the coast of Hong Kong and wanted to know if the rock-pools contained any seaweeds, if any sublittoral algae were present and if the bare rocks carried any microscopic plants.

Miss CHIU replied that the rocks, the rock-pools and part of the sublittoral zone were completely bare but she had not looked for microscopic forms on the rocks. Fertile *Sargassum siliquastrum* had been observed in October and an intact specimen of *Turbinaria ornata* was found in September, so she felt this was a field waiting for a great deal of research to be done.

To a question from Dr. ISAAC, Miss CHIU answered that the *Sargassum* and *Turbinaria* were probably growing on the rocks and were presumably completing their life cycles during the other half of the year, on more exposed shores outside the harbour.

THE ECOLOGY OF *Gracilaria confervoides* (L.) Grev. IN SOUTH AFRICA WITH SPECIAL REFERENCE TO ITS ECOLOGY IN THE SALDANHA-LANGEBAAN LAGOON

By Wm. Edwyn Isaac (South Africa)

ABSTRACT

Gracilaria confervoides is abundant in the Saldanha-Langebaan Lagoon, situated off the southern part of the west coast of South Africa. The area is about sixteen miles long consisting of a northern part with deeper water and in more direct contact with the open sea, and a southern part, ten miles long, shallower from 0·85 to 2·5 miles wide and with a number of sandbanks. *Gracilaria* is cast up at various points along east coast of the lagoon to extent of about a thousand tons air dry weight per annum which forms basis of a small agar industry.

The greatest proportion of *Gracilaria* occurs at about two feet below L.W.S.T. level, extending upwards to just above L.W.S. and downwards to about ten feet below L.W.S., although material has been dredged from greater depths. Plants occur on sandbanks, anchored in deeper water and floating a little above the bottom. Plants with basal attaching discs are known to occur only on the concrete slipway at Langebaan: in shallow water beyond L.W.S.T. level.

In the southern part of the lagoon *Zostera capensis* and *Gracilaria confervoides* are the dominant species. They do not enter into serious competition since *Zostera* characteristically occurs in shallower water and grows on a sand-mud mixture while *Gracilaria* grows on sand.

On sandbanks and in deeper water *Gracilaria* plants lack attaching discs and are weighted down by sand and/or by entanglement especially with mussels. Cystocarpic plants occur throughout the year and tetrasporic plants for most of the year. Plants possibly grow in a free floating condition. It was shown that fragments of such plants grow in a characteristic way in culture.

INTRODUCTION

Although many seaweed genera show a wide geographical distribution, the great majority of seaweed species have a relatively limited geographical distribution. *Gracilaria confervoides* (L.) Grev. is one of a very small group of species which show such a wide distribution that they can be referred to as cosmopolitan.

There are phycologists who are suspicious of the validity of widely distributed species. Certainly *G. confervoides* shows a wide range of variation in size, colour, anatomical structure and ecological behaviour as MAY (1948) has clearly shown for Australian plants of the species. As yet, however, no convincing evidence has been presented for regarding the plexus of forms included under one specific name as a group of closely related species.

It may be pointed out that in 1950, PAPENFUSS put forward a case for substituting the name *Gracilaria verrucosa* (Huds.) PAPENFUSS for *G. confervoides* (L.) Grev. (PAPENFUSS, 1950) For the present, however, the well known name for the species has been retained especially as *G. verrucosa* does not seem to have come into general use in the post 1950 literature.

Gracilaria confervoides is present in sufficient amount on the east coast of Australia (WOOD, 1946) and on the southern part of the east coast of North America (TSENG, 1947) to be exploited as a source of agar and it is used as a supplementary source of agar in Japan (TSENG, 1947). In South Africa it forms the basis of a small agar industry. (ISAAC and MOLTENO, 1953).

MORPHOLOGY

South African *Gracilaria confervoides* has a terete, filiform and usually much branched thallus which is generally of a brown or dark red colour. Plants which are directly attached to a firm substratum have a basal attachment disc. The recorded length to which the species grows differs in different parts of its distribution range. The South African plant would seem to attain greater lengths than the Californian plant which grows to a length of 90 cm or 3 ft. (DAWSON, 1945) and is certainly larger than the British plant which is described by NEWTON as being up to 50 cm (about 20 inches) long (NEWTON, 1931). The lengths attained by South African plants are not on the other hand, as impressive as those recorded for Australian (WOOD, 1945) and British Columbian (PILLSBURY, 1950) plants. On the coast of British Columbia the plants, according to PILLSBURY, average 1·6 m in length but often attain lengths of 3–4 m. In the vicinity of Beaufort, North Carolina on the eastern seaboard of U.S.A., the plants average 0·2 to 0·5 m reaching one metre in extreme cases (CAUSEY *et al.*, 1946). I have collected South African plants up to 185 cm (about 74 inches or

over 6 ft.) but it is not claimed that this is either a maximum or frequent length although a majority of plants would seem to be over a metre long. MAY has delimited four forms among Australian plants of *G. confervoides* and has shown that to some extent length and width are form characteristics (MAY, 1948).

The vegetative structure of the plant body is the same in male, female and tetrasporic plants but in general the male plants are of smaller size. The cystocarpic or mature female plants are easily distinguished as they bear dome-like prominences on the surface of the thallus. In section, filamentous cells were noted extending from the sterile region of the gonimoblast to the inner wall of the cystocarp which feature distinguishes *G. confervoides* from *G. Sjoestedtii* which it otherwise closely resembles (DAWSON, 1945; MAY, 1948). There are no surface protuberances to mark the site of tetraspores and spermatia.

Plants obtained from Hout Bay (Fig. 1) sometimes show a fasciation with a resulting marked local flattening of the thallus. A

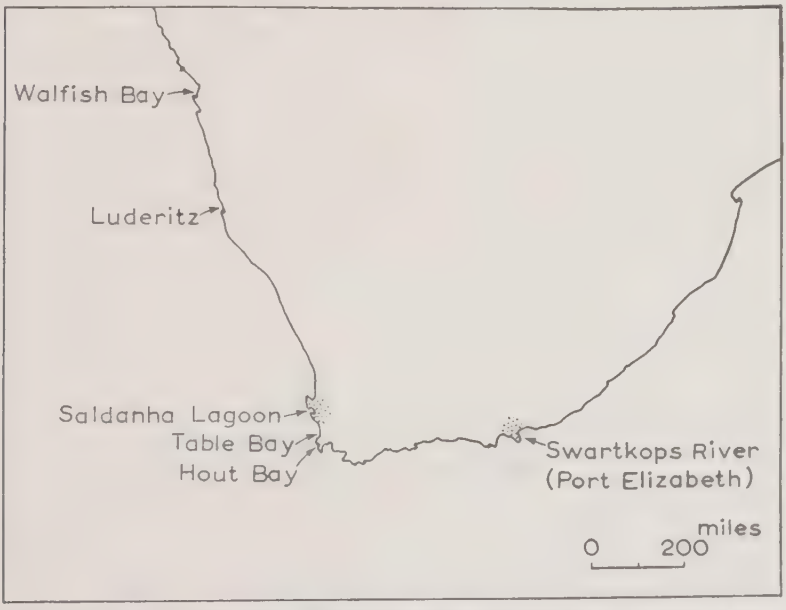


FIG. 1. Map of Southern Africa with stippling where *Gracilaria confervoides* occurs. The species has also been recorded in the other localities indicated.

similar condition has been illustrated by MAY for Australian plants (MAY, 1948, Plate 1, Fig. 2).

DISTRIBUTION ON SOUTH AFRICAN COASTS

On the west coast of Southern Africa, *Gracilaria confervoides* has been recorded from Hout Bay, Table Bay, the Saldanha-Langebaan Lagoon and has been reported from Luderitz (Fig. 1). To the north of the Union it has been recorded from Walfish Bay.* Since the Table Bay records were made (by TYSON) there have been extensive harbour works and land reclamation activities in Table Bay and the Rogge Bay locality is now dry land.† Fair amounts of *Gracilaria confervoides* occur in Hout Bay but the submerged beds in this locality have not been located. Periodically, large amounts of the plant are cast up on the shore of Hout Bay. Fishermen also report that they bring up the plant in their nets from time to time. As far as is at present known, however, *G. confervoides* occurs in very large amounts only in the Langebaan-Saldanha Lagoon where it occurs in great profusion. This region yields about 1000 tons, on an air dry-weight basis, of cast up weed per annum. This represents about six to seven thousand tons fresh weight of cast up material.

Until the last few years *Gracilaria confervoides* was thought to be confined to the west coast of South Africa and thus to be a member of the South African cold-water flora since the west coast is subject to a slow moving current of cold water of sub-antarctic origin. Subsequently it has been recorded‡ cast up on the banks of the lower reaches of the Zwartkops river near Port Elizabeth. This locality is situated in a region of moderately warm water since the east and south coasts as far as Cape Agulhas are influenced by the warm Agulhas current which arises in the tropical waters of the Indian Ocean (ISAAC, 1937a). The quantities of cast up weed present, the general trend of the current systems around South African shores, the distance from the west coast localities and the absence of records of cast up *Gracilaria confervoides* in the intermediate

* The Tyson collection of South African seaweeds in the Bolus Herbarium, University of Cape Town, includes an unnumbered sheet (LEGIT W. TYSON 1894) with Table Bay given as a locality. On the same sheet there is a specimen of a carposporic plant with a pencilled annotation 'Walfish Bay'. According to Mr. N. S. PILLANS, who has made a careful study of the handwriting of South African botanical collectors, the writing on the sheet in question is that of Tyson.

† The Rogge Bay specimen is marked Tyson No. 51 and is dated 1908.

‡ It was first recorded by Dr. M. A. POCOCK and Dr. W. MACNAE and later by the Author and Dr. W. MACNAE.

region make it unlikely that this material has come from any great distance to the west of Port Elizabeth. This record can thus be accepted as representing a very considerable extension of the known range of the species on South African coasts.

Although *Gracilaria confervoides* has been recorded over a considerable geographical area in Southern Africa the number of locality records within this area is small. This is hardly surprising when it is remembered that this plant seems to flourish only in habitats where special conditions occur. Of these, the absence of heavy seas is the most important and heavy seas prevail over large parts of the South African coasts.

THE SALDANHA-LANGEBAAN LAGOON

The Saldanha-Langebaan Lagoon is situated off the west coast (Fig. 2) and can be regarded as a small more or less inland sea. It has a maximum length of about 16 miles. The northern part is more exposed to the open sea and the waters are here deepest although eastwards of a line joining Houtjies Point and Salamander Bay, the depth is mostly less than nine fathoms. The bottom is partly rocky and partly sandy. The southern section is of a more complex character. For the greater part of its area the sea at low water spring tides has a depth of less than one fathom and only in a few places does it exceed three fathoms. Locally, however, it may range from four to seven fathoms. The bottom consists of sand or of a mixture of sand and mud, the mud component being more evident towards the southern end. The uneven depth is related to the occurrence of sandbanks between which run narrow channels of deeper water. This makes navigation difficult even in small fishing boats. This difficulty is increased by the strong, fast running tidal currents which can frequently be seen to carry a fair amount of suspended sand. This southern part has a length of somewhat over ten miles and a width which ranges from about 0·85 to a little over two and a half miles.

Most of the salinity determinations for both the northern and the southern ends gave a value somewhat above 35·5°/$_{oo}$. Further, there was no consistent difference in salinity between the northern and midsouthern ends. No water samples were collected from the southernmost part of the lagoon.

The work reported here is mostly concerned with the southern

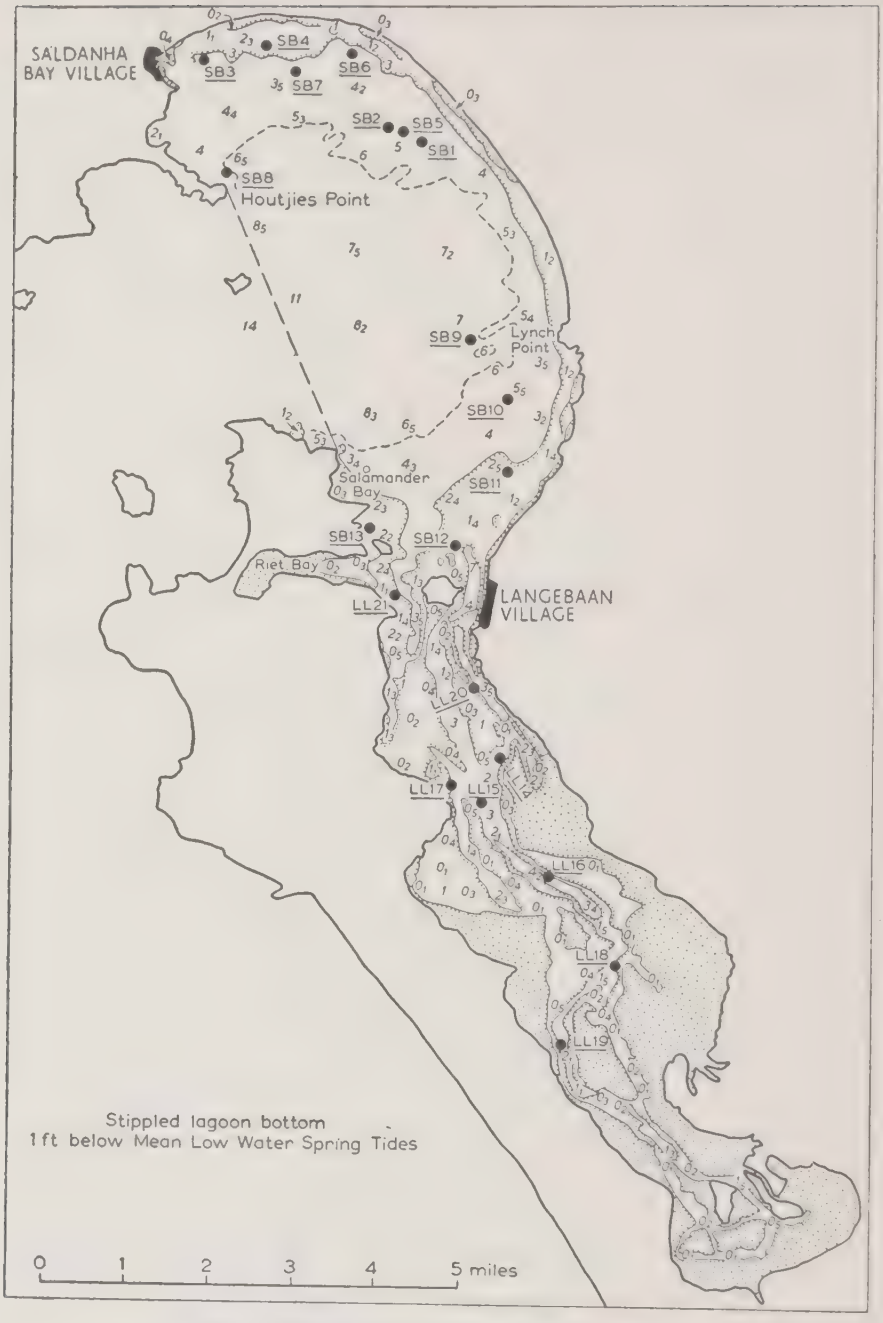


FIG. 2. The Saldanha-Langebaan lagoon.
Houtjies Bay lies north and east of Houtjies Point (the heavy black dots indicate stations at which dredgings for *Gracilaria* were made).

arm of the Saldanha-Langebaan Lagoon but unfortunately no sea temperature data are available for the southern end. The mean annual temperature at the northern end is 14·8° C and is higher than off the old Cape Town pier, Robben Island or Dassen Island (Isaac, 1937b). As compared with the open waters of the west coast the seasonal variation in temperature at the northern end is greater and the maximum temperatures in particular are higher (Isaac, 1937b). It may be remarked that the Saldanha-Langebaan Lagoon is the only place on the west coast where *Sargassum heterophyllum* (Isaac, No. 343) has been found growing; it having been found at the lowest intertidal level south of Langebaan and also in the sublittoral in the southern arm of the lagoon.

The Occurrence of Gracilaria confervoides *in the Saldanha-Langebaan Lagoon*

In this area *Gracilaria confervoides* is found cast up on beaches, on sandbanks and in deeper waters, and attached to a hard substratum by means of attaching discs. Plants attached to a solid substratum by means of a disc have so far only been found on the concrete slipway of the South African Air force crash station at Langebaan, where the plants are fairly common in shallow water below low water level of spring tides.

Cast up material—The exact position, extent and total amount of cast up weed varies a good deal. It has been found cast up in large amounts on the northern, north eastern and along the northern part of the east coast of the long southward arm of the lagoon. It is cast up at irregular intervals, chiefly it would seem in relation to heavy winds, especially the south east winds of summer (October–end of March). The cast up material shows all colour graduations from dark red to greenish-brown or very pale yellow (completely bleached) depending on how long the cast has lain on the beach. A bank of cast up weed can be seen from the colours of its constituent material to consist of one, two or more casts. After very heavy winds a certain amount of sea bamboo (*Ecklonia maxima*) is found mixed in with the cast up *Gracilaria*.

Plants on sandbanks and in deeper water—Sandbanks with a growth of *Gracilaria confervoides* are a feature of the narrow end to the south of the outlet to the open sea.

From July 15–20 1943 the beds of *G. confervoides* on sandbanks

and in deeper waters from Langebaan southwards were mapped (Fig. 3). In this part of the area there are two prominent and dom-

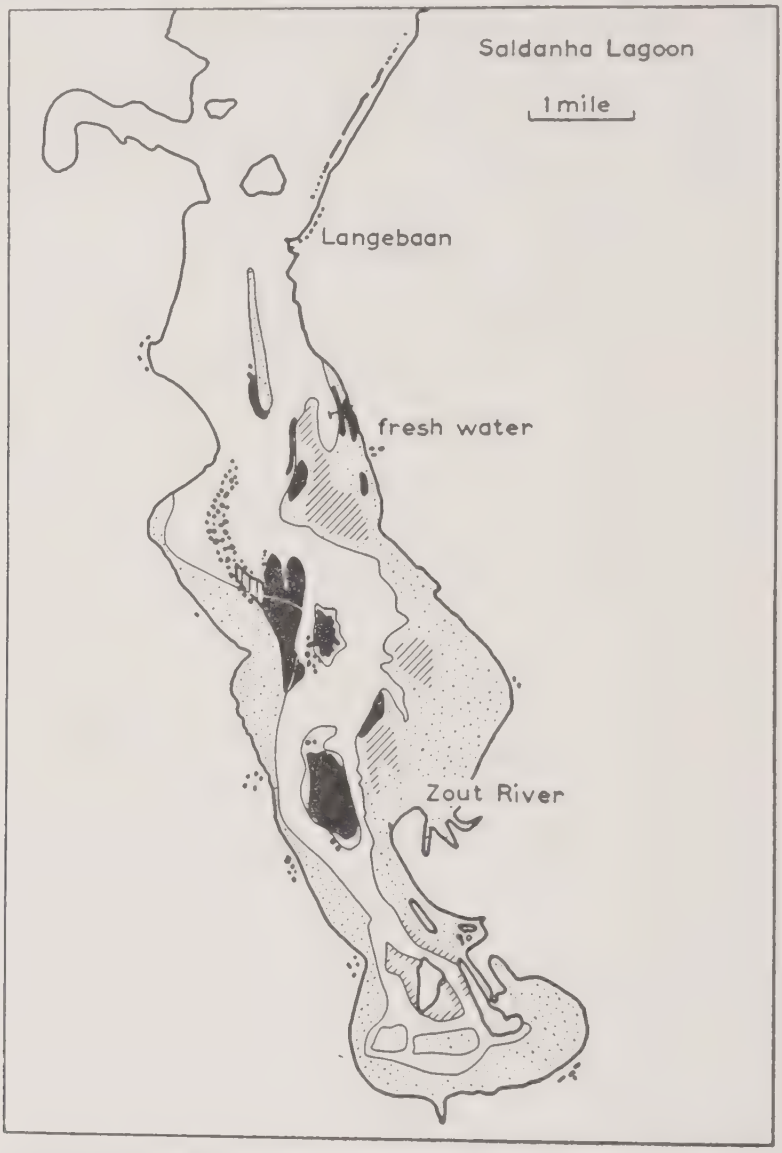


FIG. 3. Beds of *Gracilaria confervoides* (darker areas) and *Zostera capensis* (lighter areas) in the southern arm of the Saldanha-Langebaan lagoon. (Surveyed in July 1943.)

inant species; *Gracilaria confervoides* and *Zostera capensis*. The two species characteristically occur at different vertical levels and thus

do not enter into direct competition with one another to any extent. Usually there is not only a difference in vertical level but also in the character of the substratum on which the two species occur for while *G. confervoides* is found growing on sand, *Z. capensis* is found growing on a sand-mud mixture.

The greater proportion of the area occupied by *Gracilaria* in the southern part of the lagoon lies at a depth of about 2 ft. below the level of low water spring tides (L.W.S.) and thus about 7 ft. below high water level of spring tides (H.W.S.).

At the upper extreme small 'outcrops' extend to just above L.W.S. and small isolated beds extend downwards to depths of 8–9·5 ft. below L.W.S. but as pointed out later dredging operations indicate that to some extent the plant grows also at greater depths. *Zostera capensis* on the other hand, is mostly exposed at low water of spring tides, and this species extends over a considerable area of the sandbanks. It occurs at a depth of about 4 ft. at H.W.S.

The map is to be regarded essentially as indicative of the position and extent of the beds of *Gracilaria confervoides* and *Zostera capensis*. Considerable care was taken with the survey but it is clear from subsequent observations that the amount of *Gracilaria* present varies from year to year and at different times of the year.

The plants on the sandbanks become large and luxuriant. The anchored and free floating thalli are typically dark red in colour. Paler red and brownish coloured plants also occur which are usually of smaller size. The plants occur in practically pure stands and are mostly without epiphytes.

During part of the year (middle and late summer) young black mussels (*Mytilus meridionalis*) are found on the plants to a sufficient extent to lessen severely the value of the cast up weed for a few months in the year.

The most puzzling feature of these plants is the apparent habit of 'rooting' in sand without attachment to a more solid substratum. Both in 1943 and especially in 1946 a certain amount of digging was done to ascertain whether the *Gracilaria* plants were attached to more solid bodies such as pebbles, shells, worm tubes, etc., buried in the sand. It was only possible to dig in very limited areas for limited times since by far the greater part of the *Gracilaria* beds are not uncovered at low water spring tides. Where digging was possible the sand seemed to be singularly free of shells, pebbles

etc., except for localised mussel beds. Further, although many plants were examined no evidence of an attaching disc was found. Many of the *Gracilaria* plants float freely near the sandy bottom. Those that become anchored appear to become so in one of two ways and sometimes by a combination of both. Most commonly, plants would seem to become fixed by the accumulation of sand over a part of the thallus. In this way part of the thallus may be buried at a depth of six or more inches. Plants are also weighted down by attached mussels.

On the coast of British Columbia, PILLSBURY (1950) records the occurrence of sterile and tetrasporic plants of *Gracilaria confervoides* anchored by being looped around the tubes of a marine annelid worm, *Diopatra ornata* Moore.

In July 1946, dredging was carried out in the northern and eastern part of Houtjies Bay and in the shallow arm of water to the south with the object of searching for plants of *Gracilaria confervoides* attached by basal discs to stones, shells etc. Although *Gracilaria* was found in various places no plants with basal discs were found. In places an abundant growth of *G. confervoides* was found at the northern end of Houtjies Bay at depths of up to 20 ft. Again there was an abundant growth at a depth of up to 18 ft. just north of Riet Bay. At other stations little or no *Gracilaria* was found. It was not recorded at depths greater than 27 ft. below mean water level and plants found in water deeper than 20 ft. were small and moribund.

The Growth of Detached Fragments of Gracilaria confervoides

It has been shown that in North Carolina loose, drifting purely vegetative plants of *G. confervoides* continue to grow (HUMM, 1944). This material is the basis of the agar industry of eastern North America. Attached plants occur in insufficient quantity to be of economic importance. There is no direct evidence available that growth of floating plants takes place in the Langebaan-Saldanha Lagoon. This possibility, however, cannot be excluded in view of the considerable amount of *G. confervoides* cast up annually. A few culture experiments were carried out to demonstrate that growth of free floating detached fragments could possibly occur in this area. Material was collected from freshly cast up plants on the beach and again of plants floating near a sandbank in the lagoon. The axis

FIG. 4. Regeneration in culture of lateral branches from pruned 2 inch lengths of *Gracilaria confervoides*.

A

B

C

FIG. 5. Regeneration of a single pruned fragment of *Gracilaria confervoides* in culture between June 18 and November 7. (A. Original fragment.)

was cut into about 2 inch lengths and any branches present were drastically pruned to very short lengths. These plant fragments were kept in filtered sea water in a laboratory in Cape Town. The water was changed once and sometimes twice a day.

Growth was of a very characteristic type (Fig. 4). The main axis did not increase in length but new branches arose at intervals. These small new branches formed characteristic whorls at the upper ends of the axis lengths as well as at the upper ends of any old branch stump. Examination of cast up material shows plants with this kind of growth, i.e. growth of branches formed secondarily from detached fragments of older plants.

The rate of growth obtained under laboratory conditions was very slow. This is illustrated by a series of photographs of one fragment of thallus over the period June 18 to November 7, nearly five months (Fig. 5). It is realised that since the fragments of *Gracilaria confervoides* were not grown under controlled conditions, under a range of conditions and with material collected at different times of year, the results have no critical value in regard to rate of growth. The experiments, however, demonstrate clearly that free floating and freshly cast up fragments are able to grow.

Note on Annual Reproductive Cycle of Plants Growing on Sandbanks off Langebaan

In the Langebaan-Saldanha area well developed cystocarpic plants can be found at most times of the year in the mounds of cast up plants and among those growing on the sandbanks.

A monthly batch of plants was examined from a sandbank off Langebaan for a complete year (May 1952–April 1953) with a view to ascertaining:

(*a*) whether there were fertile plants of some kind to be found throughout the year,

(*b*) the relative abundance of sexual to tetrasporic plants.

An arbitrary unit of not less than 50 cm (20 inches) with branches arising at intervals of at least 5 cm (2 inches) was adopted as representing a plant. A length of 50 cm is less than a third of the known maximum length attained by the plants. In the case of non-cystocarpic plants, sections were cut at 12·5 cm (5 inches) intervals along the main axis and its branches. The results obtained are of indicative value only since the number of plants

examined varied from month to month, the samples were too small, the records extend over one year only and the minimum size unit chosen to represent a plant possibly tended to exclude the smaller male plants.

Three conclusions, however, can be drawn from the results:

(*a*) Cystocarpic plants occur throughout the year.
(*b*) Tetrasporic plants occur for at least the greater part of the year.
(*c*) The greatest proportion of plants throughout the year are carposporic.

ACKNOWLEDGEMENTS

Thanks are due to Dr. D. R. HARPER for help in carrying out the survey of the *Gracilaria* and *Zostera* beds in July 1943 and for drawing the original map on which Fig. 3 is based. The examination of monthly batches of *Gracilaria confervoides* for tetrasporic, male and female plants was done by my research assistant, Mr. R. H. SIMONS, to whom thanks are due. Lastly, it is a pleasure to acknowledge the financial support of the C.S.I.R. which made it possible to carry out certain aspects of this work.

REFERENCES

CAUSEY, N. B., PRYTHERCH, J. P., MCCASKILL, J., HUMM, H. J., and WOLF, F. A. (1946): Influence of Environmental Factors upon the Growth of *Gracilaria confervoides*, *Duke Univ. Marine Station, Bull.*, **3**, 19.

DAWSON, E. Y. (1945): An Annotated List of the Marine Algae and Marine Grasses of San Diego County, California, *San Diego Soc. Nat. Hist., Occasional Papers*, 7.

HUMM, H. J. (1944): Agar Resources of the South Atlantic and East Gulf Coasts, *Science*, **100**, 209.

ISAAC, WM. EDWYN, (1937a): South African Coastal Waters in Relation to Ocean Currents. *Georg. Rev.*, **27**, 651.

(1937b): Studies of South African seaweed vegetation. 1.—West coast from Lamberts Bay to the Cape of Good Hope. *Trans. Roy. Soc. S. Afr.*, **25**, 115.

ISAAC, WM. EDWYN and MOLTENO, C. J. (1953): Seaweed Resources of South Africa, *J. S. Afr. Bot.*, **19**, 85.

MAY, VALERIE, (1948) The Algal genus Gracilaria in Australia. *Division of Fisheries, Rept.* 15, *C.S.I.R. Bull.* 235.

NEWTON, L., (1931): *A Handbook of the British Seaweeds*, London.

PAPENFUSS, G. F., (1950): Review of the Genera of Algae described by Stackhouse, *Hydrobiologia*, **11**, 181.

Pillsbury, R. W., (1950): Natural anchoring of the red alga, *Gracilaria confervoides* (L.) Grev. on unstable bottom by association with an annelid worm. *Canad. J. Res.*, **28**, sec. C., 5, p. 471.

Tseng, C. K., (1947): Seaweed Resources of North America and Their Utilization, *Economic Bot.*, 1, p. 69.

Wood, E. J. Ferguson, (1945): The Sources of Agar in Australia, *J. Coun. Sci. Industr. Res. Aust.*, **18**, 263.

(1946): Agar in Australia, *Coun. Sci. industr. Res. Aust., Bull.* 203.

DISCUSSION

Dr. Newton, England, remarked that in spite of the wide distribution of *Gracilaria confervoides* she was not able to detect any specific difference between specimens from different parts of the world. She also commented upon the extraordinary length of the plant in Australia and the fact that it was found in low salinity water such as Swan river and Sidney Middle Harbour.

Dr. Aleem, Egypt, said that he had found specimens of *Gracilaria confervoides* 6 feet long near Alexandria and "infected" with annelid worms.

STUDIES IN SEAWEED POPULATIONS OF ECONOMIC IMPORTANCE IN DIGBY NECK AREA, NOVA SCOTIA

By Constance I. MacFarlane (Canada)

Read by E. Conway (Scotland)

Since 1948 the Nova Scotia Research Foundation has been engaged in studies of seaweeds of commercial importance at various places along the coast.

This account deals chiefly with a littoral survey of Digby Neck and its island extensions to the southwest and briefly also with its continuation northeastward along the Bay of Fundy.

The promontory of Digby Neck and its two extensions, Long and Brier Islands, are 65 miles long and 2 to 3 miles wide. They are situated in western Nova Scotia and separate the Bay of Fundy proper from St. Mary Bay, an inlet of the Fundy. With its remarkably straight shoreline and tremendous tides it is one of the most interesting coastal regions in eastern North America.

The tides of the bay have long been famous and until 1954 were the highest on record. Because of the shape of the bay the tidal differences are progressively greater as one approaches the narrowing head of the bay, and due to the direction of the earth's rotation are higher on the Nova Scotia Shore than on the New Brunswick Shore. At Yarmouth, a short distance inside the mouth of the bay, the tidal range is 10 ft. at neaps to 16 ft. at highest springs. At the head of the bay, in Minas Basin, the range is 46 ft. at neaps to 53½ ft. at highest springs.

Not only is the Fundy noted for its tides, but its southeast coast from Brier Island to Cape Split is a strip of shoreline unique to Nova Scotia and to the Atlantic Coast of North America. Like the Palisades of the Hudson River and the inland formation further south, it is formed of Triassic trap with overlying layers of softer sandstone. The erosion of the superficial layers has left a long straight shoreline on the southeastern side of the bay with a few

shallow coves and three straits, Digby Gut, Petit Passage, and Grand Passage.

This hard ridge, extending from Cape Split in the northeast and sloping downward toward the southwest, runs under the sea at the southern tip of Brier Island to form dangerous ledges 2 fathoms deep running 2 miles southwest of Gull Rock. Shoals $2\frac{1}{2}$ miles beyond these are the site of heavy tide rips and laminarians.

In Digby Neck itself and its island extensions the basalt ridge is double with a valley of amygdaloid trap in the centre. At each end of Brier Island the valley is drowned by the sea to form Pond Cove in the southwest and Westport Harbour in Grand Passage. The opposite side of the passage has a similar cove on Long Island. At each side of the coves are rocky capes of basalt.

The region presented peculiar difficulties for littoral surveys. There are few low water harbours on this stretch of coast and because of high cliffs it is not possible to reach the shore at all points by land. The area from Brier Island to Delap Cove was surveyed in August and September 1951 and June 1952. 110 miles (35 miles in St. Mary Bay and 75 miles in the Bay of Fundy) were covered by boat when the weather permitted, and by automobile and foot when conditions were unsuitable for boat travel.

Above Delap Cove the nature of the shore and tides makes it impossible to conduct littoral surveys from a boat at low water. Consequently stereoscopic aerial photographs were made of the whole shoreline (exclusive of St. Mary Bay) in July 1952 and with their aid the remainder of the survey was completed by automobile and on foot the following spring.

The 'dip' of the ridge is northwest toward the Fundy shore resulting on that side of the neck in a broad gently shelving intertidal zone of smooth rock ledges and boulders with here and there a few areas of gravel or sand. From the tip of Brier Island to Delap Cove, 10 miles above Digby Gut, the shore bears littoral weed in spectacular abundance. From this point on towards Cape Split there are many acres of harvestable rockweed, but the areas of sand and gravel increase in frequency and extent with a corresponding decrease in weed cover. At Scotsman Bay the sea terrace is almost entirely of sand and there is no harvestable weed.

The species of rockweed found here are:

Ascophyllum nodosum (L.) Le Jol.
Fucus vesiculosus L.
F. edentatus De la Pyl.
F. spiralis L. (of no commercial importance)
F. evanescens C. Ag.

The most abundant of these is *Ascophyllum*, which in the neck and islands forms more than 80% of the rockweed cover. Further up the bay the *Fucus* increases (75% at Margaretville).

On the 68 miles of coastline from Brier Island to Digby Gut and on Gull and Grand Passage ledges it is estimated that there are 900 acres of weedbed bearing 40,000 tons of harvestable rockweed. From the Gut to Delap Cove, an additional 10 miles, there are 100 acres with 5,000 tons. The remainder of the coast, 83 miles to Scotsman Bay, supports 35,000 tons on 1500 acres. The extent and density appear in Table 1.

Table 1

Location	Distance	Area of harvestable weed	Tonnage	Variation in density (tons per acre)
Pond Cove to Digby Gut	68 miles	900 acres	40,000 tons +	57 tons to 20 tons average 40–45 tons
Digby Gut to Delap Cove	10 miles	100 acres	5,000 tons +	
Delap Cove to Scotsman Bay	83 miles	1500 acres	35,000 tons	39 tons to 8 tons average 20–25 tons
Totals:	161 miles	2500 acres	80,000 tons	

The heaviest densities were found in Brier Island at Hogyard Cove and Bar, Pond Cove (1200 tons) and near Western Light; in Grand Passage on Cow Ledge (600 tons), and on Long Island ledge nearby. The plants, except in a few favored locations such as Pond Cove, are more exposed to wave action than in the Cape Sable-Wedgeport area and are generally shorter, but often with stronger holdfasts and more numerous branches.

If conservation of the beds is to be maintained it is important that the plants be harvested in such a way that at least six or seven inches be left attached. If this is done harvests can be obtained from the same area once in four or possibly three years. Where the *Ascophyllum* is cut back too severely or pulled from the substratum, it is replaced by *Monostroma* and *Fucus vesiculosus*.

Gigartina stellata (Stack.) Batt. occurs in extensive beds between the fucoid and laminarian zones. The density varies from 1 to 3 lbs. per square foot and the plant is easily harvested at low water of spring tides. It grows either in pure stands or mixed with *Corallina*, *Ulva*, *Monostroma*, *Fucus*, *Plumaria* and *Rhodymenia*. It should be raked rather lightly so as not to damage the holdfasts. Excessive raking leads to the intrusion of these other weeds and the depletion of the beds.

Several railway carloads of *Gigartina* could be harvested annually from Digby Neck and islands. Further up the bay the density decreases.

Because the *Gigartina* is fully exposed only at low water springs and because it could not be distinguished as a genus in the black and white aerial photographs no attempt was made to assess the tonnage.

Although not in harvestable quantities, another intertidal alga growing profusely in the area is *Plumaria sericea* (Harv.) Rupr. Elsewhere in the Province it occurs in much smaller quantities and usually in the wash or attached to stones or shells among the holdfasts of *Laminaria* and on *Phyllophora*. In the Digby Neck region it is everywhere abundant in both bays growing on vertical rock faces under the overhanging fronds of fucoids and in other dimly lit places. In the *Gigartina* beds it frequently forms a short turf beneath the *Gigartina*.

The shoreline of St. Mary Bay is in sharp contrast to that of the Fundy proper. Here the 'strike' of the ridge is exposed and the greater part of the western side of the bay is lined with steep basalt pillars. The intertidal zone is narrow and rugged and the shore falls away quickly into deep water. The intertidal weeds are insufficient for commercial harvesting and for the most part are attached to massive rocks tumbled down from the pillars above, a substratum too rough for harvesting operations.

The only algae of commercial importance on the southeastern

side of the Neck are laminarians. In order of abundance these are:

Laminaria longicruris De la Pyl.—from a short distance below L.W. to 5 fathoms and occasionally lower—throughout the width of the zone. Plants are usually large. Specimens were taken up to 23 ft. 4 ins. long and 33 ins. wide.

L. digitata (L.) Edmondston—near L.W. and down to 5 or 6 fathoms, but most abundant in shallower depths.

Alaria esculenta (L.) Grev.—at low water level.

Agarum cribrosum (Mertens) Bory—regularly forms a fringe at the deeper edge of the *Laminaria* belt (4–7–10 f.). This plant occurs here in much greater numbers than in other parts of the Province which have been examined.

Phyllaria dermatodea (De la Pyl.) Le Jol.—found occasionally in St. Mary Bay and more frequently in the swifter waters of the Passages and Gull Ledge.

These kelps, the chief of which is *L. longicruris*, grow in an almost continuous belt 10–60 yds in width (av. about 30 yds.) from the tip of Brier Island to East Sandy Cove, a distance of 20 miles.

It was estimated that 8,000–10,000 tons of kelp occur in this strip.

Small dense beds of *Laminaria* occur along Petit and Grand Passages where the water is clear and the currents swift (5–6 knots in Grand Passage; 7 knots in Petit Passage). The submarine ledges beyond the southwest tip of Brier Island also have a dense kelp cover, particularly of *Laminaria digitata* and *Alaria esculenta*. Southern Ledge, about two miles beyond, supports a heavy growth, but because of the shoal (2 fathoms) and uneven rocky bottom and the uncertain tide rips it was not possible to sample it satisfactorily. It is only occasionally that favourable harvesting weather occurs on these ledges.

Laminarias are found from these ledges to Brier Island and in closely adjacent patches all the way along the Fundy shore to Digby Gut and a short distance beyond. The patches are sometimes in coves and sometimes on ledges extending out from points between the coves. The plants are usually attached to rock and are almost never found on shells or stones as in the Tusket-Cape Sable area. Although many patches exhibit dense growth, this unsheltered shore offers no regular opportunites for harvesting.

Frequent storms add to the scouring effect of the tidal current which carries silt from Minas Basin. The currents are not easily disturbed by wind and are as strong at a depth of 30 fathoms as at the surface. After storms there is often little left in shallow water but the holdfasts and basal parts of the stipes.

Because kelps of harvestable density in this region are limited to shallow waters with strong tidal currents, and confined chiefly to a narrow zone bordering the coast, it was not possible in the time available to assess the tonnage in the Bay of Fundy.

CYTOLOGICAL OBSERVATIONS ON THREE BRITISH SPECIES OF *Laminaria*; A PRELIMINARY REPORT

By Margaret Naylor (England)

The three species investigated were *Laminaria digitata*, *Laminaria ochroleuca* and *Laminaria saccharina*. Plants were grown in culture from zoospores and nuclear divisions followed in the gametophytes of both sexes and in the young sporophytes. In all three species the structure of the nucleus is the same, as is the sequence of phases in nuclear division.

The resting nucleus is granular in appearance, usually with one or two granules larger and more deeply staining than the rest, and possesses a conspicuous nucleolus. During prophase the nucleus enlarges considerably and the chromosomes differentiate as fine, beaded threads. These undergo drastic condensation into short, stout rods and finally into spherical bodies about 0·5μ in diameter. A widely spaced prometaphase stage is followed by a normal compact metaphase plate and anaphase separation of two plates of daughter chromosomes.

Separation of the chromatids begins during prophase and is achieved by an equatorial constriction which divides the chromosomes into two equal parts. During prophase the whole structure remains approximately spherical, but in the metaphase plate the side view of the chromosome is elongated and dumbbell-shaped. During early anaphase the elongation increases and the isthmus joining the two halves becomes drawn out into a fine thread and eventually ruptures. The two uniform plates then pass to the poles remaining approximately parallel the whole of the way. No U-, or V-shaped curvature of separating chromatids is seen; this presumably is mechanically impossible in such small chromosomes. In the early stages of anaphase one or two bridges are usually present. After their rupture the chromosomes concerned lag slightly behind the rest of the plate.

Formation of two daughter nuclei, and wall formation follows in

the normal fashion in both gametophytes and sporelings. There is no indication that 'multiplication of the chromosomes occurs whilst dispersed in the cytoplasm', as described by Walker (1954) in *L. digitata*, or that 'endomitotic multiplication to polyploidy' occurs during the first divisions of the sporeling.

A number of chromosome counts have been made on all three species, usually at the prometaphase stage in the division prior to oogonium formation in the female gametophyte, since at this stage there is very considerable enlargement of the nucleus. The values obtained for *n* have not been consistent, and in *L. digitata* range from 27 to 33; in *L. ochroleuca* from 25 to 30; and in *L. saccharina* from 27 to 31. These figures are sufficiently close to indicate that in all probability, with improved techniques a definite count will be obtained.

These counts are very much higher than any others previously recorded either in the genus *Laminaria* or within the Laminariales. The discrepancy between the results in the present paper and those of Walker (1954) and Magne (1953) on the same species is probably due to the inadequacy of the methods yet used for such small chromosomes.

REFERENCES

Walker, F. T., (1954): Chromosome Number of *Laminaria digitata*, *Ann. Bot. N. S.*, **18**, 112.

Magne, F. (1953): Meiose et nombre chromosomique chez les *Laminariaceae*, *C. R. Acad. Sci., Paris*. **236**, 515.

DISCUSSION

Dr. Newton, England, asked whether Dr. Naylor had any difficulties in fixation and if the temperature played any part in this process. Dr. Naylor stated that temperatures of -10° C retarded fixation as compared with room temperature; hot fixation had not been attempted.

Dr. Braarud, Norway, stressed the impotance of a clear taxonomy of the *Laminarias* from the point of view of exploitation and had hoped that the chromosome numbers would be of some help. Dr. Naylor's work indicated that no such help could be expected.

RECUPERATION AND RECOLONISATION IN *Ascophyllum*

By Henrik Printz (Norway)

Read by E. Baardseth (Norway)

The usual method of collecting *Ascophyllum* is to cut the whole seaweed cluster with a sickle or scythe at the base, leaving only short stubs a few centimetres in length. It has been assumed that the remaining stubs would sprout, but experience has shown that this does not always occur. An inspection of areas harvested at different times indicated that the stubs had apparently to a large extent vanished.

Further, it was observed that in areas which formerly had been covered by a dense growth of *Ascophyllum*, no new population had immigrated after harvesting, even after a lapse of several years.

In order to investigate this phenomenon more closely I carried out some harvesting experiments in the summer of 1952. The results of these experiments may be summarised as follows: In areas with 25 cm stub height there was already quite a satisfactory regrowth in the following year. Admittedly the plants were only 30–35 cm long, but they showed an abundance of new shoots. The decapitated main stem, having lost its points of growth, had not increased in length. Two years after the cutting the areas gave the impression of being almost completely restored.

The regrowth in the areas which had been cut to 15 cm was somewhat less satisfactory, while the area which had been cut to 5 cm stub height stood practically unaltered.

Comparisons of photographs taken at intervals of one year during the last three years show that there is practically no change discernible except that some tufts have disappeared here and there (Fig. 1).

From these observations it appears that the low stubs left after harvesting of *Ascophyllum* possess no or only a very modest power of recuperation. It seems that only the shoots which were so short that they escaped decapitation have the power of continuing their

growth, but they remain, at any rate during the first years after the cutting, short, for the most part unbranched and decidedly more slender than is normally the case.

With regard to the practically complete lack of recuperation from the stubs, it is natural to suppose that the more mature lower parts of the plant also have a correspondingly lowered assimilation intensity.

In order to determine the photosynthesis in shoots of various ages an investigation was carried out in the Oslofjord. Assimilation declined gradually with advancing age, but even the oldest shoots examined, which in this case were 8 years old, showed a positive oxygen balance, at any rate in the summer. Lack of essential assimilatory products can therefore scarcely be the real hindrance to recuperation. The cause of this must be looked for in other factors, probably a stimulus emitted from an active embryonic tissue, which presumably initiates the development through growth-substances.

It is interesting to note that wholly similar phenomena occur in *Fucus*, according to the investigations by Knight and Parke in 1950.

Apart from the regular dichotomic ramification of the long main shoots, all normal new formation in *Ascophyllum* occurs from the marginal grooves which are so characteristic of this genus. These are formed bilaterally on the thallus directly from the apical points of growth and contain embryonic cells. *Ascophyllum* seems to lack the power of forming true adventitious shoots, i.e. new formation from fully grown tissues. Thus all secondary formation on a shoot normally originate from these pits. But their embryonic tissue has only a limited period of life, and its activity gradually declines with age. A natural consequence of this successive extinction of embryonic activity are the naked, sterile and unbranched basal stems, which can be seen on all older individuals of *Ascophyllum*.

Recuperation after harvesting will accordingly depend on whether any functional points of growth, apical or lateral, exist on the remaining stubs.

With our present knowledge, there is no doubt that it would be a far more suitable method of harvesting to pull up plants, when the aim is to insure a speedy and effective regeneration. By this method only the longest and oldest shoots will be harvested, leaving the younger and shorter ones. The cutting will have the character of a

thinning and by this procedure full advantage will be taken of the more effective photosynthesis and growth of the young shoots.

With respect to recolonisation in *Ascophyllum*, it is a remarkable fact that in some cases no new population of sporelings appears in harvested areas. However, as the lifetime of the individuals is limited, it is clear that sooner or later the invasion of a new population must ensure. According to my experience during recent years, such a repopulation apparently does not occur every year. Because *Ascophyllum* is extremely fertile every year, it must be assumed that some special combination of climatic conditions is needed for an effective recolonisation.

In nature, recolonisation of *Ascophyllum* will be balanced by the relatively long lifetime of the individual plants—on our coasts 15 to 20 years or even more. By harvesting, however, the original equilibrium is disturbed and difficulties of recuperation and repopulation are manifest.

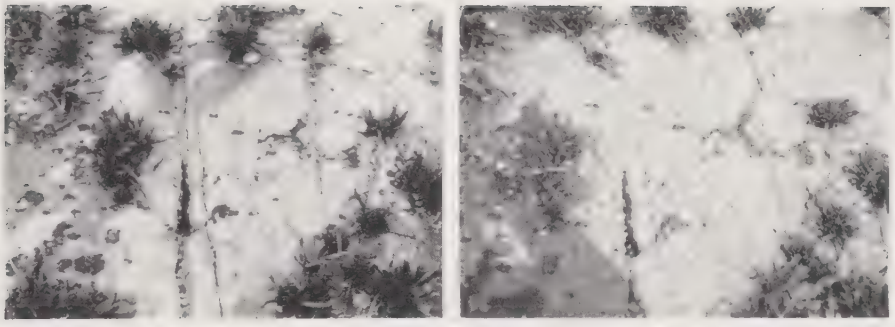
Knight and Parke (1950) mention that '*Ascophyllum* did not regenerate on itself on the cleared areas during the period of the experiment. Sporelings appeared in small numbers and reached a length of a few cm but inevitably disappeared again. Even at this date, 8 years after the original clearance, there is still no sign of the establishment of an *Ascophyllum* population'.

Also from the French coast, where perhaps the littoral algae are most heavily exploited, except for Norway, DE VIRVILLE (1953) reports that in localities where seaweed is collected the algal vegetation is on the decline. It is stated that large areas already lie bare of algal vegetation, and in many places the Fucaceae have entirely disappeared. The situation is regarded as serious and sharp measures are called for to prevent indiscriminate harvesting in the future from leading to the destruction of a natural resource 'qui n'est certainement pas négligeable'.

The difficulties of regeneration which have manifested themselves in the cropped areas, at any rate as regards *Ascophyllum*, seem, therefore, to be a general phenomenon.

REFERENCES

KNIGHT, M. and PARKE, M., (1950): A Biological Study of *Fucus vesiculosus* L. and *F. serratus* L., *J. Marin. Biol. Assoc. United Kingdom*, **29**, 439–514.



a *b*

FIG. 1. (*a*) *Ascophyllum*, one year after cutting.
(*b*) The same field three years after cutting.

Virville, Ad. Davy de, (1953): Dépeuplement de la flore marine sur les côtes occidentales du Cotentin, *Proceed. First Inter. Seaweed Symposium*, Edinburgh, pp. 26–28.

DISCUSSION

Mr. Richardson, Scotland, mentioned that similar observations had been made in Scotland: no regrowth occurred in two years when the plants were cut close to the base. Recolonisation by *Fucus* species was observed in some cases, and complete regrowth of *Ascophyllum* which was cut 8–10 inches from the base took place within two years. Dr. Chesters had made similar observations on the Yorkshire coast and had found a full recolonisation within 3 years.

Dr. Newton mentioned that she had reached exactly the same conclusions from similar experiments.

Mrs. Kylin replied that *Ascophyllum* showed a tendency to disappear from the Swedish coast, the beds being invaded by *Fucus* species.

Mr. Flood mentioned that Dr. Baardseth had carried out similar investigations in Ireland, and he wanted to know how these results agreed with those obtained by Dr. Printz. Mr. Flood further remarked that extensive use of sickles led to very poor regrowth contrary to the method of tearing away the weed by hand which resulted in rapid recolonisation by the smaller plants left undamaged.

Dr. Baardseth stated that the regrowth generally depended upon the method of harvesting, cut to the base it was very difficult for *Ascophyllum* to recolonise the bed. To a question by Mr. Flood, Dr. Baardseth stated that he had found the best of *Ascophyllum* to be approximately 5 years. To a question by Mr. Flood, Dr. Baardseth verified that he had found the best regrowth period of *Ascophyllum* was cut 15 cm from the base the plants would take 4 or 5 years to reach full size.

Mr. Flood asked about the influence of local conditions such as height of tides and velocity of currents etc. on the rate of growth.

Dr. Baardseth said that Irish specimens seemed to grow more vigorously than Norwegian ones.

PHENOLOGY OF *Ascophyllum nodosum*

By Henrik Printz (Norway)

A convenient species for phenological studies in algae is *Ascophyllum nodosum*. In this species a new vesicle is formed at the top of each shoot in the spring, and the distance between the vesicles indicates therefore each year's growth and production. Moreover, according to the number of vesicles it is possible to determine the age of the individuals.

It has been found on the coast of Norway, at any rate, that the length of the annual shoot increases from the spring and during the course of the summer and autumn, until about November-December, while during the darkest part of the winter there is almost complete stagnation. But at a fairly early date, in South Norway at the beginning of February, the first signs of increased activity are visible at the top of the shoots. This is indicated by the fact that the shoot tips assume a lighter colour and gradually begin to swell. A section through the tips in these first stages reveals that they consist of quite normal cellular tissue, but gradually, as the apical swelling increases in size, the cellular walls in this zone become looser and brighter, and the whole cellular tissue gradually becomes more mucous. When the apical swelling has attained approximately the size of a millet-seed the mucilage is gradually resorbed, and a small central gas-filled cavity is formed, which quickly grows in size. At this stage it is possible by squeezing the tip to feel unmistakably that it is beginning to be gas-filled. Thus the apical vesicles arise and all the above-mentioned phases can be dated quite easily and afford a good means of determining the stage of development attained.

Further, it becomes fairly easy to determine the time when a small apical tip begins to form on the young vesicle, and to see how this new shoot increases in length during the course of the summer and autumn.

Another easily determinable phase occurs when the conceptacles begin to appear near the top of the receptacles. This stage is ascertained most easily by the aid of a magnifying glass in translucent

light. The conceptacles are then seen as small globular dark dots. A further characteristic stage occurs when the oogonia begin to divide. When the receptacles mature it is easy to see the difference between the male and female plants, the receptacles of the male plants being more yellow in colour, while the female ones retain their original tint. Moreover, the conceptacles are usually somewhat larger in the female and have larger openings.

When the gametes are released the empty receptacles soon disintegrate, and are shed at a fairly quick rate within a comparatively short and easily determinable time.

New receptacles appear immediately after the old ones are shed, in some cases even a little before. This phase of development is also suitable for observation, as are the phases when the new receptacles have reached a length of 5 and 10 mm respectively.

In order to ascertain to what extent the annual development in *Ascophyllum* depends on the degree of latitude, material from various places along the Norwegian coast, namely Fevik, Kopervik, Espevær, Kristiansund, Horsvær, Reine, Ingøy, and Vardø, has been regularly examined (Fig. 1). The results for the year 1952 are shown in Table 1.

Table 1.

	First sign of new vesicles	First sign of tip outside vesicle	Period of development in days	Conceptacles become visible	First division of oogonia	Spermatogonia developed	Receptacles shed	New receptacles 5 mm long	New receptacles 10 mm long	Period of development in days
Fevik	10/2	20/4	70		1/4		1/6	1/6	15/6	15
Kopervik	15–20/2	25/4	65–70	25/2	1/4		5/6	5/6		
Espevær	20–25/2	1/5	65–70			1/4	5/6	10/6	20/6	10
Kristiansund N.	10–15/3	1/5	46–50			1/4	5–10/6	10/6	20/6	10
Horsvær	5/4	15/5	40	5/4	1/6	5/5	20–25/6			
Reine	20/4	20/5	30		1/6		25/6	5/7		
Ingøy	30/4	25–30/5	25–30			5/6	5–10/7	10/7	20/7	10
Vardø	5–10/5	5–10/6	ca. 30				10–15/7	10/7	20/7	10
Time difference in number of days between Fevik and Vardø	85/90	46/51					40/45	40	35	

From this it will be seen that at Fevik, which is the most southerly station, the first signs of vesicle formation at the top of the shoot appear on approximately February 10. Following the development from station to station northwards we find that at Kopervik and Espevær the same phase occurs about 1 to 2 weeks later, at

FIG. 1.

Kristiansund about 1 month later, and continuing northwards the delay in time becomes steadily greater, until at the most northerly stations, Ingøy and Vardø, it is 80–90 days. Another phase of development, which has been determined, occurs at the time when the first shoot appears at the top of the vesicles. This took place at Fevik about April 20. It is possible to follow the delay in development northwards up to Vardø, where the same phase emerges between June 5 and 10, that is to say a delay in time of 45 to 50 days.

The time which elapsed from the appearance of the new minute vesicle until a small shoot forms on top of it was approximately 70 days at Fevik, but further northwards the period becomes constantly shorter, until at Vardø the development takes only about 30 days, or less than half the time at Fevik.

With regard to the development of the reproductive organs, the date when the conceptacles are first visible in the magnifying glass

as small dark dots in the tops of the receptacles has been studied, and also the date when the oogonium first begins to divide and the spermatozoids are released. With respect to these sexual stages of development we have, however, only a few perfectly trustworthy observations for 1952, but they show a delay of the date of maturity according to the degree of latitude, which corresponds very well with that of the vegetative parts.

The fertility period closes with the shedding of the receptacles. At Fevik this occurred at the beginning of June, at Vardø toward the middle of July, i.e. a difference in time of 40–45 days. At Fevik the new receptacles had attained an average length of 5 mm by about June 1, and at Vardø by about July 10, i.e. 40 days later. At Fevik the receptacles were 10 mm long about June 15, at Vardø they reached this length on July 20, 35 days later. The growth from 5 to 10 mm took approximately 15 days in the first half of June at Fevik, while at Vardø, in the middle of July, it took only 10 days.

Summarizing the results it may be said, in the first place, that the whole process of development is retarded corresponding to the increasing degree of latitude. This is a perfectly natural and obvious course of events, but it is of interest to know exactly how great the delay in development is in the various localities along the coast—in other words to ascertain the regularity of the delay.

There is another and very interesting feature which emerges, namely that during the spring and summer the development in the northern regions takes place at a relatively greater pace than in the southern regions, and at an increasingly quicker rate the farther north we move. In this way the considerable difference in the development, amounting to nearly 90 days, which is found during the late winter and early spring, constantly diminishes, until at midsummer it is not more than about 30 days.

Investigations were continued in the years 1953 and 1954, and mainly from the same stations. But the summer of 1953 was in meteorological respects very abnormal, being reported to be the sunniest summer for 70 years in the most northerly counties, Nordland, Troms and Finnmark. At the same time South Norway had a great excess of precipitation, and with a correspondingly thick covering of clouds. During the whole autumn of 1953 the weather in the North was clear and calm. This abnormal type of weather had consequences also for the development of the marine algae.

The phasic aberration proceeded in that year in general as in 1952, up to midsummer, but in the north the development, owing to the exceptionally sunny summer, continued at such a rapid pace that it largely caught up with, and in some cases overtook, the development in the south.

But, what is still more curious, in the following summer too, 1954, the weather was approximately the same as in 1953, with a quite unusually sunny summer in North Norway and an equally wretched and rainy season in the South. I have not yet managed to work up the material for these years, but I hope to have an opportunity of doing so and presenting the results later.

DISCUSSION

Dr. NAYLOR, England, suggested that the phenological phenomena found by Dr. PRINTZ could be due to a photo-periodic effect. She had herself been growing *Laminaria* in cultures, some with 12 hours day and 12 hours night and some with continuous illumination, and had obtained differences in growth.

Dr. BURROWS, England, wanted to know the number of plants on which Dr. PRINTZ's observations were based. Dr BAARDSETH said he did not know, but he had himself collected from the Reine locality samples of 4–5 kg for Dr. PRINTZ.

Dr. BRAARUD, Norway, said that a knowledge of the phenology of the seaweeds was very important in connection with the seasonal variation in chemical composition. He also remarked that this investigation would have to continue for a long time because the weather differs greatly from year to year.

Dr. ISAAC, South Africa, remarked that all too often marine ecologists in the past have neglected for the intertidal algae the analysis of the land climate which he thought very important in a consideration of the distribution of algae over a great range of latitudes and with different sea temperatures.

PERENNIAL CHANGES OF *Laminaria cloustoni* Edm. (*L. hyperborea* (Gunn.) Fosl.) AROUND SCOTLAND

By W. D. Richardson and F. T. Walker (Scotland)

Read by W. D. Richardson

Part of the investigation into perennial changes which may occur in crops of *Laminariaceae* has been carried out in three widely separated areas of Scotland where the dominant species is *Laminaria cloustoni*. The areas are separately described below.

Girvan to Bennane Head, S.W. Scotland

The survey area, 16 km long, is off the coast of Ayrshire and forms part of the eastern boundary of the approaches to the Firth of Clyde.

The hinterland for the most part rises gently from a sandy foreshore. The seabed is chiefly one of boulders and sand with some areas of rock and gravel.

The offing stream, setting easterly through the North Channel after passing the Mull of Kintyre, expands and its north-easterly part strikes Bennane Head; one part flows north-east at a rate of 2·5 knots (4·63 km/hr) during spring tides. The offing stream setting in a northerly direction through the North Channel meets the south-easterly stream out of the Firth of Clyde off Bennane Head. The sub-littoral zone of the survey area is fed by the incoming Atlantic and outgoing Clyde waters; thus marine salts will be enriched with estuarine effluents, while ocean salinity will be diluted by land drainage.

Four sub-littoral surveys have been carried out in this area for the present investigation during the periods 23 April to 25 May, 1952, 24 March to 13 May, 1953, 9 March to 21 April, 1954, and 14 March to 15 April, 1955.

The four surveys were completed before the advent of the 'May cast', when the old laminae of *Laminaria cloustoni* are shed.

The method and rate of random sampling were the same in each

of the four surveys (WALKER, 1947, WALKER and RICHARDSON, 1955). The area was divided into 17 subareas, each approximately equal to 900 metres of coastline; 1700 quadrats were measured in each survey from low water mark down to a depth of 6 fathoms (11 metres).

The density, cover and calculated quantity of *Laminariaceae* (based on all quadrats) over 1881 acres (761 hectares) from low water down to 5 fathoms found for each survey are given in Table 1.

Table 1

Survey year	Mean density fresh weight (ton/acre)	Cover %	Quantity (tons)
1952	18·1	75	34,000
1953	10·9	61	20,000
1954	10·4	60	19,600
1955	11·6	68	21,900

1 ton/acre = 2500 kg./hectare; 1 ton = 1016 kg.; 1 acre = 0·4047 hectare.

Comparison of the total quantities of seaweed shows that of the 34,000 tons found in 1952, 40% was lost one year later. Most of this loss was known to have occurred during a period of storms in September 1952 (WALKER and RICHARDSON, 1955).

By 1954 a further loss (4%) was found but by 1955 a gain (10%) on the 1954 value had occurred.

The density and cover were further analysed to show the differences at various depths of one fathom intervals, Table 2.

Table 2

Depth (fathoms L.W.M.O.S.T.)	Mean Seaweed Density, Based on all quadrats, fresh weight (lb./sq.yd.)				Seaweed Cover %			
	1952	1953	1954	1955	1952	1953	1954	1955
0	9·0	7·0	5·0	6·5	62	62	62	68
1	11·6	8·6	8·6	8·6	79	72	75	78
2	12·6	8·7	7·9	8·6	86	75	74	82
3	9·1	5·0	6·3	5·7	80	63	71	73
4	6·2	1·9	2·6	3·1	76	54	61	62
5	3·7	1·0	0·7	1·0	69	40	38	43
6	2·6	0·8	0·6	0·5	58	37	39	32

1 lb./sq.yd. = 0·54 kg./sq. metre.

Laminaria cloustoni increased its dominance especially in deeper water.

Dunbar to Fast Castle, S.E. Scotland

The survey area extends for a distance of 18 km from Dunbar south to Fast Castle, west of St. Abbs Head. The coast has a straight-forward configuration and forms part of the eastern boundaries of East Lothian and Berwickshire. The coast is exposed to the North Sea and during rough weather, waves break with considerable force. The hinterland slopes gently to the sea and a similar gradient continues underwater to 37 metres, although in the region of St. Abbs Head steep cliffs occur. Along the coast sandy bays are found where quantities of sublittoral algae are cast up during certain seasons of the year.

The strata of the seabed is folded, the folds running out at right angles to the coast.

The tidal stream during spring tides along this coast is 1 knot (1·85 km/hr) in the neighbourhood of Dunbar but increases near St. Abbs Head. The tidal range during equinox spring tides is 6 metres.

Three sublittoral surveys have been carried out in this area for the present investigation, during the periods 1 September to 14 November 1949, 1 July to 21 August 1952 and 20 July to 24 September 1954.

The method and rate of random sampling were the same in each of the three surveys.

The area was divided into 19 subareas each approximately equal to 900 metres of coastline; 1900 quadrats were measured in each survey from low water mark down to 7 fathoms (13 metres).

The density, cover and calculated quantity of *Laminariaceae* over 1840 acres (744 hectares) from low water down to 5 fathoms found for each survey are given in Table 3.

The quantity of *Laminariaceae* decreased (59%) from 1949 to 1952 but recovered by 30% from 1952 to 1954.

The density and cover were further analysed to show the difference at various depths of one fathom intervals in Table 4.

Table 3

Survey year	Mean Density fresh weight (ton/acre)	Cover %	Quantity (tons)
1949	19·1	91	35,200
1952	7·8	65	14,400
1954	10·2	67	18,700

1 ton/acre = 2500 kg./hectare; 1 ton = 1016 kg.; 1 acre = 0·4047 hectare.

Table 4

Depth (fathoms L.W.M.O.S.T.)	Mean Seaweed Density based on all quadrats, fresh weight (lb./sq.yd.)			Seaweed Cover %		
	1949	1952	1954	1949	1952	1954
0	12·4	3·9	8·2	97	72	86
1	11·1	5·6	7·5	96	75	82
2	10·0	4·8	5·9	95	74	79
3	8·7	4·1	4·1	95	69	71
4	7·1	2·7	2·5	92	61	56
5	3·5	1·3	0·8	73	37	28
6	1·2	0·5	0·1	49	20	8
7	0·4	0·1	0	33	6	2

1 lb./sq.yd. = 0·54 kg./sq. metre.

There is evidence that loss of cover between 1949 and 1952 was caused by increased deposits of sand in the area.

Laminaria cloustoni remained the dominant species at all depths.

Fraserburgh to Rosehearty, N.E. Scotland

The survey area lies between Clubbie Craig north-west of Fraserburgh, and the Bay of Lochielair 7 km to the westward and 1·5 km west of Rosehearty. The coast is low lying and forms the eastern part of the southern coast of the Moray Firth. The area is fully exposed to the North Sea and to north-easterly weather.

The tidal streams during spring tides average less than 1 knot (1·85 km/hr). The tidal range during autumnal equinox spring tides is 4·6 metres.

Three sub-littoral surveys have been carried out in this area for the present investigation during the periods 16 September to 8 October 1952, 31 August to 24 September 1953 and 5 October to 1 December 1954.

The method and rate of random sampling were the same in each of the three surveys.

The area was divided into 7 subareas each approximately equal to 1000 metres of coastline; 700 quadrats were measured in each survey from low water mark to a depth of 10 fathoms (18·5 metres).

The density, cover and calculated quantity of *Laminariaceae* over 724 acres (293 hectares) from low water down to 5 fathoms found for each survey are given in Table 5.

Table 5

Survey year	Mean density fresh weight (ton/acre)	Cover %	Quantity (tons)
1952	14·1	87	10,200
1953	8·3	62	6,000
1954	14·4	85	10,400

1 ton/acre = 2500 kg./hectare; 1 ton = 1016 kg.; 1 acre = 0·4047 hectare.

The quantity of *Laminariaceae* decreased from 1952 to 1953 by 41 % but recovered completely by 1954.

The density and cover were further analysed to show the differences at various depths of one fathom intervals in Table 6.

Laminaria cloustoni remained the dominant species in all depths.

Table 6

Depth (fathoms L.W.M.O.S.T.)	Mean Seaweed Density based on all quadrats, fresh weight (lb./sq.yd.)			Seaweed Cover %		
	1952	1953	1954	1952	1953	1954
0	4·2	2·5	7·8	75	62	88
1	5·6	4·9	6·5	84	67	89
2	8·3	4·7	6·7	93	64	87
3	7·0	3·7	7·1	88	60	82
4	7·3	4·0	6·6	92	63	90
5	6·5	2·5	3·7	90	57	72
6	4·3	1·5	2·8	90	41	61
7	2·2	1·0	2·9	72	42	56
8	1·2	0·4	1·9	56	25	48
9	0·3	0·2	1·0	25	14	38
10	0·3	0·3	0·7	19	21	20

1 lb./sq. yd. = 0·54 kg./sq. metre.

Conclusions

The following conclusions can be drawn from the data which is available to date.

1. There have been considerable perennial changes in the standing crop of *Laminaria cloustoni* in the three areas investigated.
2. In each area decreases in quantity have been followed by varying degrees of recovery.
3. In each area the decreases occurred at all depths. Subsequent increases were found in shallow water at Girvan and Dunbar, but at all depths at Fraserburgh.
4. *Laminaria cloustoni* remained the dominant species in the three areas during the periods of investigation.

REFERENCES

Walker, F. T. (1947) Sublittoral Seaweed Survey, *J. Ecol.* **35**, 166–185.

Walker, F. T. and Richardson, W. D. (1955) An Ecological Investigation of *Laminaria cloustoni* Edm. (*L. hyperborea* Fosl.) around Scotland. *J. Ecol.* **43**, 26–38.

DISCUSSION

To a question from Dr. Newton about the methods used Mr. Richardson answered that they had used a spring grab covering 0·5 m² for sampling *Laminaria cloustoni*. They took 100 quadrats per km² and distributed the samples as evenly as possible between low water mark and six or ten fathoms.

Dr. Aleem asked Mr. Richardson about the efficiency of the spring grab. Mr. Richardson replied that the spring grab obviously left some of the smaller plants behind and he did not know exactly what proportion of the weed was taken. He pointed out that the quantitative values obtained were probably less than the amounts which would be available to industry. Dr. Aleem wanted to know where the plants were usually cut, close to or far above the holdfast. Mr. Richardson said that the plants were in general cut just above the base.

Mr. Flood remarked that the figures for *Laminaria cloustoni* were rather high and wondered whether a variety of *Laminaria digitata* might be admixed. Mr. Richardson said that the possibility was ruled out because special beds had been selected where *L. cloustoni* was dominant and with only negligible amounts of *L. digitata*.

Dr. Newton mentioned that the selection of certain beds for the investigation would hardly influence the reliability of the method, and this was verified by Mr. Richardson.

Mr. McInnes said that in his opinion very few plants escaped the spring grab.

Dr. PERCIVAL wondered if Mr. RICHARDSON had some further information on the changes observed. Mr. RICHARDSON said that between 1947, when the sublittoral survey was started, and 1952 there has been general decreases in density in many areas. Some increases in density had been recorded since 1952. It was thought that strong gales were a major factor in bringing about the initial decreases in density.

Dr. SØRENSEN pointed out that control experiments undertaken by Dr. BAARDSETH had shown that the spring grab took from 30% to 65% of what it was supposed to take (see Report No. 6 from Norwegian Institute of Seaweed Research).

Dr. WOODWARD mentioned that the investigation presented by Mr. RICHARDSON formed part of an extensive 10 year programme of the Scottish Seaweed Research Institute and that at least 100,000 samples had been taken by spring grab which had been dropped 100,000 times into the sea.

Dr. ALEEM wondered if this spring grab was available to other people than the Scots and Norwegians. Dr. WOODWARD answered in the affirmative.

THE RESISTANCE OF *Gracilaria confervoides* TO HYDROGEN SULPHIDE

By K. Stokke (Norway)

Gracilaria confervoides Grev. was first recorded in Norway in 1935, from the southwestern coast (Levring, 1937). In 1945 it was recorded from the Oslofjord (Grenager, 1947) and from 1948 to 1955 it has been observed at a number of localities. Its present northern limit lies at the latitude of Trondheim. South of Stad it is common in suitable localities all along the coast (Fig. 1).

The alga is usually sterile and unattached. At one locality only was it found to be fertile, viz. on a rocky shore near Lindesnes, the southernmost point of Norway. Its distribution is restricted to semi-closed bays, where the water temperature in summer reaches 16–20° C. The localities where *Gracilaria* occurs in abundance are characterized by having muddy bottom with H_2S in the mud. For one locality hydrographical observations are available. In Fig. 2 is shown the borderline between H_2S-water and the upper aerated layer at this locality throughout the year. During winter the H_2S-water approached the surface and flooded the *Gracilaria* beds. The following year aeration was more effective and conditions for algal growth more favourable.

The vegetation of this locality consists mainly of *Gracilaria* with a minor component of *Ceramium sp.* and *Furcellaria fastigiata*. *Gracilaria* occurs at a depth of 1 to 6 m, with the largest quantities at 4 m. Below 6 m no algal vegetation was observed.

The *Gracilaria* vegetation is here exposed to water containing H_2S (presumably less than 5 ml./L) during a certain part of the year. The plants were more slender than at localities with aerated water all year round.

In order to check the resistance of *Gracilaria* to H_2S-water simple experiments were carried out in the laboratory. The alga is easy to culture and it grows within a large range of salinity. The hardy nature of this species is illustrated by the fact that a specimen which was kept in the dark at $+3°$ C for a whole year still showed

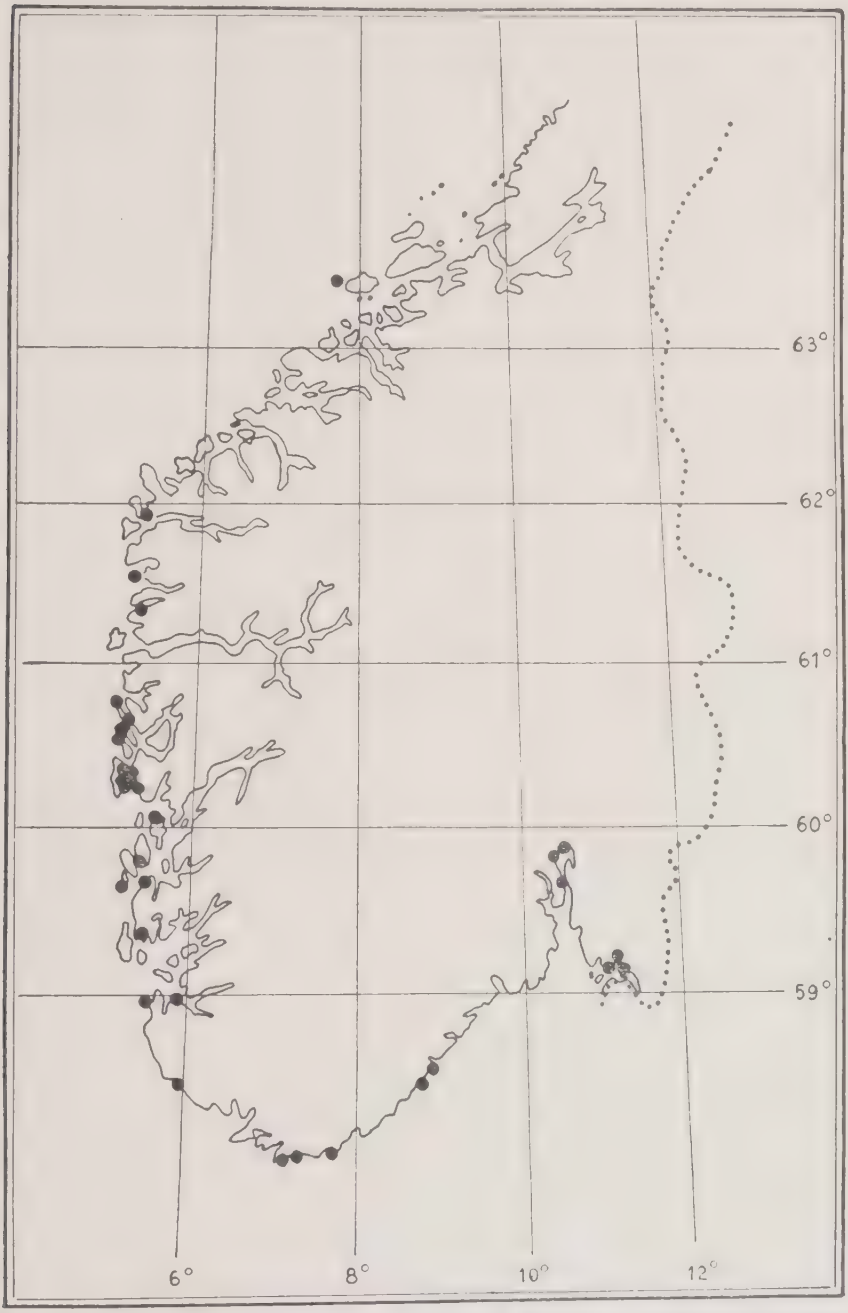


FIG. 1. Localities where *Gracilaria confervoides* has been recorded in Norway.

good growth when transferred to light and favourable conditions for growth.

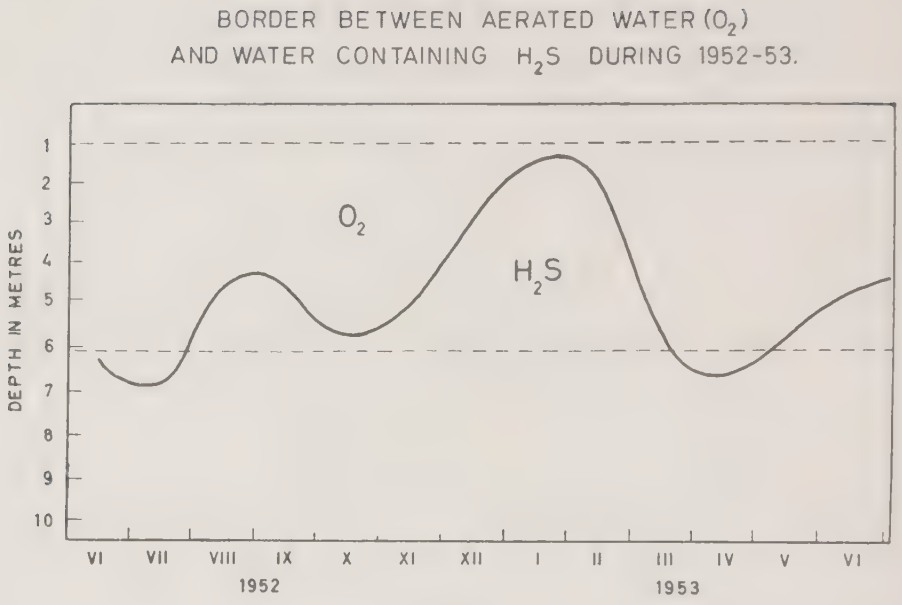


FIG. 2.

The H_2S-experiments were carried out with whole plants in dark bottles with sea-water of different H_2S-concentrations at a temperature of $+3°$ C. Higher temperatures were not used since the H_2S-concentration then varied too much. After the exposure to H_2S-water the plants were divided into 2 cm long pieces which were transferred to ordinary sea-water and kept at conditions presumably favourable for growth.

The results of the experiments are presented in Fig. 3. On the abscissa are indicated the number of days of immersion and on the ordinate the concentration of H_2S in ml./L. The dotted horizontal line indicates the approximate level of the H_2S content in many land-locked waters in Norway. Dark points indicate that the alga was alive after the treatment, crosses that the specimens were dead. Most of the dead specimens were infected with fungi.

The observations indicate that for a short period of time the alga can survive at high concentrations of H_2S. At concentrations of about 5 ml./L the plants were able to survive immersion for 40 days in the dark, the temperature being $+3°$ C. These conditions are comparable with the situation in the bay mentioned above, where the H_2S-water invaded the *Gracilaria*-beds during winter time.

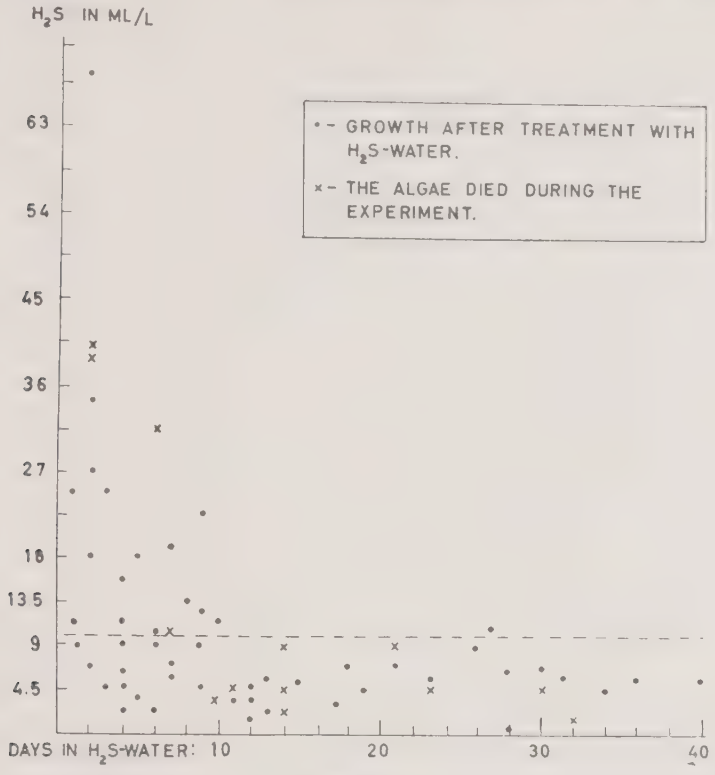


FIG. 3.

Detailed studies of the reaction of the alga upon treatment with H_2S at conditions more favourable for growth (higher temperature and ample light supply) were not undertaken.

REFERENCES

GRENAGER, B., (1947): Orienterende undersøkelser over algevegetasjonen i indre Oslofjord og dens avhengighet av forurensning. (Manuscript)

LEVRING, T., (1937): Zur Kenntnis der Algenflora der norwegischen Westküste, *Lunds Univ. Årsskrift, N. F. Avd.* 2, Bd. 33, Nr. 8, Lund.

DISCUSSION

On a question from Dr. ISAAC, South Africa, Mr. STOKKE gave the additional information that *Gracilaria* was usually found between 5 and 30 feet depth.

Dr. CONWAY, Scotland, suggested that *Gracilaria confervoides* must be tolerant to wide changes in salinity conditions.

Dr. NEWTON, England, agreed to that and told about the occurrence of this species in brackish water lakes in Gipsland in Australia. On another

occasion, she had found it twisted around the roots of mangroves in Brisbane.

Mr. STOKKE remarked that he had grown *Gracilaria* in the laboratory in water with salinity between 5 and 35‰.

Mr. GORDON SMITH, West Australia, said that *Gracilaria confervoides* grew in mud in the Swan River tidal estuary. This mud often smelt strongly of hydrogen sulphide. The alga suffered great variations of salinity due to the winter rainfall and summer drought. It must be subjected to pollution from city effluents entering the upper estuary.

Dr. ALEEM, Egypt, wanted to know the right name of this plant, *Gracilaria confervoides*, *G. Sjøstedtii* or *Gracilariopsis*.

Dr. ISAAC mentioned that he sectioned his specimens to make sure of the cystocarpic structure.

Dr. BRAARUD, Norway, asked whether anyone could offer an explanation for the resistance of this species to high H_2S-concentrations for such a long period, which he found very puzzling. He suggested that it may be very well sealed against H_2S. Mr. STOKKE also found *Ceramium*, *Polysiphonia nigrescens*, and *Phyllophora brodiaei* growing together with *Gracilaria* and in similar conditions. He also asked whether anyone knew anything about the anatomy of the cell wall which could explain the resistance. Dr. ALEEM found *Chaetomorpha* in similar conditions.

Dr. CONWAY asked Professor BRAARUD whether he thought the resistance was a question of sealing or of the pressure. He thought it a question of sealing, because if H_2S penetrated into the cells, one would not expect that they could stand it, but the matter has not been investigated.

Mrs. KYLIN, Sweden, remarked that several marine algae could stand a great deal of H_2S, but died at high temperatures. She and Professor KYLIN had discussed this question with Professor SKUJA who apparently found similar conditions. She also said that cultures could live for a whole year in H_2S-polluted water if the temperature was low.

Dr. WÆRN, Sweden, said that *Gracilaria* did not live in the Baltic where the salinity was as low as 0–8‰. He wondered whether *Gracilaria* could live in areas with a constant low salinity.

Further comments on the possible influence of salinity, *p*H and pollution upon the occurrence of *Gracilaria* were made by Dr. ISAAC, Dr. YAMADA, Dr. ALEEM, Mr. STOKKE and the chairman.

PROJET DE CARTOGRAPHIE DES PLANTES MARINES UTILES À L'INDUSTRIE

Par Ad. Davy de Virville (France)

Si les géologues ont réussi, depuis longtemps, à dresser des cartes sur lesquelles l'emplacement des différents terrains est représenté par des plages de couleurs variées; et plus récemment les Phytogéographes, la surface occupée par les associations végétales terrestres; par contre, les Phycologues, du moins en France, à l'exception de quelque tentatives isolées, ne s'étaient encore jamais préoccupés jusqu'ici de cartographier la répartition des Végétaux marins: malgré l'intérêt évident d'une telle réalisation.

Aussi avons-nous tenté de la réaliser, pour quelques points du littoral de France, à des échelles variées, et de représenter, par des couleurs aussi différentes que possible, les vingt formations de plantes marines que l'on peut approximativement identifier sur nos côtes.

Or nous avons pensé que ce travail devrait spécialement intéresser les Industriels qui utilisent un certain nombre de plantes marines que l'on peut à ce point de vue, classer en trois catégories:

1° Le 'Goëmon', constitué de *Fucacées* et de *Laminariacées* récoltées, comme engrais ou pour l'extraction de l'algine, telles que l'*Ascophyllum nodosum* que nous avons figuré en vert; le *Fucus vesiculosus*, en violet; le *F. serratus*, en rouge; et le *Laminaria flexicaulis*, en jaune.

2° Le 'Lichen', récolté pour la fabrication de gelées, constitué de *Chondrus crispus* ou de *Gigartina mamillosa*, représenté en brun.

3° Le 'Varech', employé à la confection d'emballage ou de matelas, constitué par les herbiers de *Zostères* (*Z. marina*), représenté en vert, avec des tirets.

Nous avons pu ainsi dresser quatre cartes pour des points du littoral atlantique français aussi différents que possible les uns des autres quant à leur exposition aux vagues ou à l'amplitude de la marée; ou encore à leur isolement insulaire: et ceci à des échelles variées, afin de rechercher le meilleur mode de représentation applicables dans tous les cas.

La Première de ces cartes, à l'échelle du 1/16.000ème, a été réalisée sur les côtes de la Manche, pour les environs du Roc de Granville, dans la Manche, où la marée présente une amplitude considérable atteignant 14 m. Elle met bien en évidence l'opposition qui existe entre la flore d'une pointe battue, avec ses ceintures de végétation étroites et irrégulières; et, au contraire, celle des plateaux rocheux abrités que la mer découvre sur une large surface pouvant atteindre plusieurs kilomètres, et qui sont recouverts de ceintures de Fucacées largement étalées, remplacées, au voisinage des basses-mers, par des banquettes de Chondrus activement récoltées.

La seconde carte, à l'échelle du 1/25.000ème, intéresse les côtes de la Presqu'ile de Quiberon, dans l'Océan Atlantique. Elle montre, mieux encore que la précédente, l'opposition encore plus marquée existant entre la côte Est abritée dont la flore est assez analogue à celle des côtes de la Manche, mais n'occupe qu'une surface beaucoup plus étroite en raison de l'amplitude moitié plus faible de la marée; et, au contraire, la côte Ouest, violemment battue, aux formations d'Algues irrégulières qui finissent même par ne plus pouvoir se développer dans les stations les plus exposées à la violence des vagues. Ici la récolte, à la différence des côtes de la Manche, devient impossible.

La troisième carte au 1/46.000ème est celle des Iles Houat et Hoëdic, isolées à 12 ou 20 km en mer à l'extrêmité de cette presqu'île. Elle souligne également la différence entre la flore d'Algues calcaires de Corallines et de Lithothamnium localisée aux pointes les plus battues et celle des Laminaires, au pied des falaises un peu moins exposées.

Enfin la quatrième carte représente la flore de la même île Hoëdic, mais réalisée cette fois à l'échelle du 1/1.000ème. Elle montre la nécessité d'utiliser une grande échelle pour établir la minute de ces cartes qui devrait être ensuite uniformément réduite, au 1/20.000ème qui, comme pour les plantes supérieures, du reste, paraît être la meilleure pour cette cartographie. Les formations d'Algues, dans ces conditions, sont plus distinctes et les différences écologiques, plus apparentes. De plus on a l'impression qu'en particulier lorsqu'il s'agit de la flore d'une petite île, la flore en est ainsi bien connue.

Il est superflu, après ces explications, de souligner l'intérêt

pratique qu'offrirait en particulier pour les Industriels, l'établissement de telles cartes qui leur feraient connaître immédiatement l'emplacement et l'importance des formations des Végétaux marins qu'ils utilisent. De plus on sait que la flore des pointes battues et des anses abritées est très différente. On connaîtrait ainsi très exactement les divers degrés d'exposition aux vagues des différents points du littoral: renseignement fort utile pour l'établissement des Pêcheries, des travaux d'art, etc.

Quant à leur intérêt scientifique, il est encore plus grand. Par exemple nous avons pu ainsi découvrir le rôle écologique de deux zones de végétation secondaires: celle des Nemalion sur les côtes battues méridionales de l'Océan atlantique et celle des Corallina-Lithothamnium-Pollicipes, localisée uniquement à la base des falaises les plus violemment battues par les vagues.

Or une telle réalisation ne présente aucune difficulté particulière avec l'aide de quelques collaborateurs faciles à former à ce travail. Elle n'exige pas de longs délais puisque, pendant un jour de marée, on peut relever la flore sur 2 ou 3 km de côte en moyenne. Le plus difficile—et le plus important—serait de relever également, bien entendu, les formations végétales sous-marines, à l'aide de plongées, de dragages et surtout de photographies ou d'observations aériennes ce qui est très réalisable puisqu'on l'a déjà au moins tenté en divers pays.

DISCUSSION

Dr. Aleem expressed his gratitude to Dr. de Virville for his excellent paper. He pointed out that Dr. Virville was first to make use of photography for the study of algal communities. He asked how Dr. de Virville in intricate cases managed to describe both the bottom conditions and the algae with his system of marks and colours. Dr. de Virville demonstrated that his system would cover most of the actual cases.

ON THE DISTRIBUTION OF SARGASSUM ON THE COAST OF JAPAN AND ITS NEIGHBOURING REGIONS

By Y. YAMADA (Japan)

So FAR about 70 species of *Sargassum* have been reported from the coast of Japan and its adjacent regions. Generally speaking, we can divide them into two groups from the distributional point of view: 'Southern types', and 'Non-Southern types'. Most species of the 'Southern type' group are of Indo-Pacific origin, being distributed in the region in question in Formosa, Loo-choo Islands, southern parts of Kyushu and Sikoku, and Kii Peninsula, and also in Hachijo Island where the warm current prevails. Therefore the presence of these species in this region seems to show their northern limit of distribution. It is also noticeable that these species belong mostly to J. G. AGARDH's subgenus *Eusargassum*. On the Japanese coast no species belonging to *Eusargassum* is found where the surface temperature of sea water descends below 10° C in winter.

Another type of species, the so-called 'Non-Southern type', are those which are restricted within the region in question, on the Japanese coast and in its adjacent waters. We can count here 30 species in all. Again it is noticeable that this group of species represents mostly ones which do not belong to the subgenus *Eusargassum*, only two species being assigned to this subgenus. The allotment to the subgenera of the species of the present group is as follows:

Subg. Phyllotrichia
- *S. piluliferum* C. Ag.
- *S. microceratium* C. Ag.
- *S. setaceum* Yendo
- *S. Yendoi* Okam. et Yam.

Subg. Schizophycus
- *S. tosaense* Yendo
- *S. patens* J. Ag.
- *S. pinnatifidum* Harvey
- *S. kashiwajimanum* Yendo

Subg. Bactrophycus
- *S. Horneri* C. Ag.
- *S. filicinum* Harvey
- *S. serratifolium* C. Ag.

S. tortile C. Ag.
S. giganteifolium Yamada
S. Ringgoldianum Harvey
S. sagamianum Yendo
S. Turneri Yendo
S. confusum C. Ag.
S. enerve C. Ag.
S. Thunbergii O. Kuntze
S. Kjellmanianum Yendo
S. Miyabei Yendo
S. nipponicum Yendo
S. hemiphyllum C. Ag.

Subg. Micracantha
S. micracanthum Yendo
S. nigrifolium Yendo
S. tenuifolium Yamada

Subg. Arthrophycus
S. racemosum Yam. et Segi
S. kushimotense Yendo

Subg. Eusargassum
S. assimile Harvey
S. crispifolium Yamada

From the viewpoint of distribution the species of this group can be divided again into 4 small groups, viz:

(1) Species group showing very wide distribution within the region in question.
(2) Species group found only in the southern parts.
(3) Species group found only in the middle parts.
(4) Species group peculiar to the Tsushima current.

(1) The species belonging to group (1) are as follows:

S. Horneri C. Ag.
S. Thunbergii O. Kuntze
S. serratifolium C. Ag.
S. tortile C. Ag.
S. enerve C. Ag.
S. Kjellmanianum Yendo
S. micracanthum Yendo

Among them *S. Horneri* and *S. Thunbergii* are most commonly found in the region in question and are recorded at Hong Kong or Formosa in the south, and in southern Kurile and Saghalien in the north, and the other species show about the same or slightly narrower distribution than *S. Horneri* and *S. Thunbergii*.

(2) The second group consists of the following species, which grow only in the southern parts of the region:

S. piluliferum C. Ag.
S. patens C. Ag.
S. pinnatifidum Harvey
S. kashiwajimanum Yendo
S. tosaense Yendo
S. setaceum Yendo
S. nipponicum Yendo
S. hemiphyllum C. Ag.
S. sagamianum Yendo
S. tenuifolium Yamada
S. giganteifolium Yamada
S. kushimotense Yendo
S. assimile Harvey
S. crispifolium Yamada.

(3) The species belonging to the third group show very narrow distribution, being found only in the middle parts of the region in question. So far, we know these species only from the middle parts of Japan. The following species belong to this group:

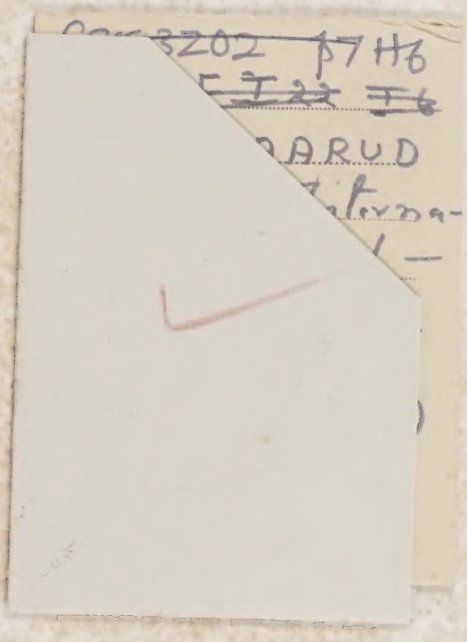
S. Yendoi Okam. et Yam.
S. Ringgoldianum Harvey
S. filicinum Harvey
S. nigrifolium Yendo
S. racemosum Yam. et Segi

(4) The fourth group contains the species which are peculiar to the Tsushima current. The warm Kuroshio current, coming from the south along the Loo-choo Archipelago puts out a branch, the 'Tsushima current', near the southern end of Kyushu, which goes northward along the western coasts of Kyushu, Honshu, and Hokkaido. There are several seaweeds which grow only in such places which are washed by the 'Tsushima current' and *S. microceratium* C. Ag., *S. confusum* C. Ag., *S. Turneri* C.Ag. and *S. Miyabei* Yendo are included in this group. They grow on the Japan Sea side of Honshu and Hokkaido and also on the southeastern coast of Hokkaido and the northeastern coast of Honshu. These two places in Hokkaido and Honshu respectively are influenced by a small branch of the Tsushima current which enters the Pacific Ocean through Tsugara strait. *Coccophora*, the monotypic genus belonging to the *Sargassaceae* is also limited to the Tsushima current.

DISCUSSION

Dr. ALEEM, Egypt, wanted to know how species of *Sargassum* could be determined. The outcome of the discussion seemed to be that they should be sent to Dr. YAMADA.

Dr. ISAAC, South Africa, mentioned that most species of *Sargassum* were warm-temperature species in South Africa, but *one* species, *Sargassum longifolium*, also extended into moderately cold water occurring together with the kelps. He also asked whether any of the Japanese species dominated intertidal communities or formed girdles and the answer was that one did, *Sargassum Thunbergii*.

CENTRAL FOOD TECHNOLOGICAL RESEARCH INSTITUTE, MYSORE. LIBRARY

Checked
Christie
15/10/57.
H 9750
Qm89
3770
CHECKED
27.5.97
CHECKED
2008
VERIFIED
2013
AC
SV2016